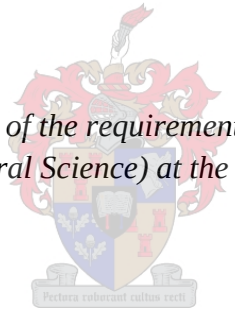
Dormancy progression, artificial rest breaking and pollination of 'Independence' almonds under South African growing conditions.

By

Tharine du Toit



Thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Agriculture (Horticultural Science) at the University of Stellenbosch

***Supervisor**:* Dr. E.D. Louw

***Co-supervisor**:* Prof K.I.T. Theron

March 2021

DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: March 2021

ACKNOWLEDGEMENTS

This thesis would not have been possible without the input and assistance of many people.

Dr Esmé D. Louw, for being open to a project that came to the department in an unconventional manner. Thank you for your support and patience throughout my studies. Your small gestures of encouragement during the editing process did not go unnoticed. Prof. Karen I. Theron, for your practical guidance and advice. Thank you for bringing years of expertise to this study, it was a privilege to have access to your insights.

I would like to thank Bertie van Zyl (Pty Ltd) t/a ZZ2 and Cape Almonds (Pty Ltd) for providing funding throughout this study and access to expert knowledge within the South African industry. To the various producers for hosting trial sites and their efforts in sampling plant material utilized, including Truter Lutz, Loutjie Hanekom, Paul de Villiers, Van der Byl Brink, Nicolas Hanekom, Richard Hewitt, Johnny Heatlie, Stephan le Roux and J.C. Coetzee.

I would like to thank Gustav Lötze and his team for their support both in the field and laboratory, I would not have been able to do this study without your assistance.

To my fellow MSc students, thank you for insightful discussions and debates regarding horticultural aspects. Thank you to A.C. Mouton, for always being prepared to assist, even with the little things. For helping me make sense of all the confusion and finding the right words to express my thoughts and ideas.

To my family, thank you for all the love and support that gave me the perseverance to complete my studies, and to my friends, for ensuring I did not neglect my social responsibilities. And finally, to Arnold, thank you for making the last two year of my time in Stellenbosch the most memorable.

Vir my ouers, Philip en Marlise, dankie dat julle my nie toegelaat het om op te skop nie.

SUMMARY

Almond (*Prunus amygdalus* Batsch) has grown in popularity with South African growers, as the need for better alternatives to marginal crops arose in the Western Cape. A failure to establish a local almond industry in the past was ascribed to limited local supply that did not justify investments in expensive machinery needed for almond processing, which further prevented the expansion of the South African almond industry. Since then, advances in almond breeding have offered a wider range of cultivars that are more suitable for production under South African conditions, such as 'Independence', a self-compatible almond cultivar characterized by a low chill requirement (400 Utah chill units) and late flowering.

Dormancy progression and bud break patterns, together with chilling and heat requirements, were investigated in various 'Independence' almond orchards grown throughout the Western Cape. A low level of dormancy was depicted for this almond cultivar, showing endodormancy progression, irrespective of chill accumulation. Dormancy induction and chill requirements varied among orchards, while the release from dormancy seemed more comparable. Results suggest that 'Independence' is more reliant on sufficient heat accumulation to ensure successful dormancy release, rather than chill. Regarding climatic conditions, 'Independence' is suitable for commercial production under South African conditions. Even though dormancy progression models showed a more acceptable representation of endodormancy release, its ability to accurately represent dormancy induction remains questionable under mild winter conditions.

Using chemical rest breaking agents (RBAs) have become standard practice in deciduous fruit production in South Africa. The efficacy of various RBAs in improving bud break and increasing possible bearing positions was evaluated on 'Independence' almond trees. None of the RBAs affected reproductive bud break, fruit set, yield and post-harvest quality parameters. However, oil containing treatments enhanced the onset of vegetative bud break, resulting in a greater overlap between reproductive and vegetative growth. As no obvious disadvantage was shown in reproductive development, earlier vegetative bud break could hold potential benefits due to an advanced photosynthetic ability. An increase in spur production was also evident in trees treated with RBAs containing oil. As almond predominantly bears on spurs, these treatments increased the bearing surface, possibly increasing yield potential in subsequent seasons. The 0.5% hydrogen cyanamide treatment, in combination with 2% mineral oil, proved

to be the most effective RBA tested to enhance vegetative growth and increase the bearing surface of 'Independence' almond trees grown under South African conditions.

The effect of commercial beehives and presence of a cross-pollinator ('Nonpareil') on fruit set and quality was evaluated. The presence of a compatible cross-pollinator did not have an effect on fruit set, yield efficiency and post-harvest quality parameters, demonstrating that single-cultivar orchards would not compromise yield potential due to a lack of cross-pollinators. Even though the epistigmatic flowers of 'Independence' almond trees have autogamic capacity, it was not efficient in ensuring maximum yield potential. The presence of pollen vectors is needed to ensure successful self-pollination and fertilization in this self-compatible almond cultivar, to obtain commercially acceptable crop loads.

OPSOMMING

Die verloop van dormansie, kunsmatige rusbreking en bestuiwing van 'Independence' amandelbome verbou onder Suid-Afrikaanse toestande.

Die gewildheid van amandel (*Prunus amygdalus* Batsch) onder Suid-Afrikaanse produsente het toegeneem weens die behoefte aan beter alternatiewe gewasse in die Wes-Kaap. Mislukte pogings om 'n plaaslike amandelindustrie in die verlede te vestig, word toegeskryf aan beperkte plaaslike aanbod wat nie die belegging in peperduur verwerkingsaanlegte regverdig het nie en verdere uitbreidings verhoed het. Sedertdien het vooruitgang in amandelteelprogramme gelei tot 'n wyer verskeidenheid kultivars wat meer geskik is vir verbouing onder Suid-Afrikaanse toestande, soos Independence, 'n selfverenigbare amandelkultivar met karaktereienskappe soos lae kouebehoefte (400 Utah koue eenhede) en later blomperiode.

Ondersoek is ingestel na die verloop van dormansie en knopbreekpatrone, asook koue- en hittebehoefte van verskeie 'Independence' amandelboorde reg oor die Wes-Kaap. Lae dormansievlakke vir hierdie amandelkultivar is uitgebeeld en dui op 'n onafhanklikheid ten opsigte van koue-akkumulasie vir die verloop van endodormansie. Alhoewel daar groot verskille tussen boorde aangedui is vir dormansie induksie en kouebehoefte, was die uitgangsproses uit dormansie meer eenvormig. Die resultate dui daarop dat 'Independence' 'n groter afhanklikheid van voldoende hitte akkumulering toon om suksesvolle dormansie-uitgang te verseker, in vergelyking met koue. Met betrekking tot klimaatstoestande blyk 'Independence' geskik te wees vir verbouing in Suid-Afrika. Daar is ook gevind dat dormansie-modelle meer geskik is vir die beskrywing van die uitgangsproses uit endodormansie, maar dat dit nie 'n voldoende verteenwoordiging van dormansie-induksie onder gematigde wintertoestande voorstel nie.

Die gebruik van chemiese rusbreekmiddels (RBMs) vorm deel van die standaard verbouingspraktyke vir sagtevrugte in Suid-Afrika. Die doeltreffendheid van verskeie RBMs vir die verbetering van knopbreek en vermeerdering van moontlike draposisies by 'Indepedence' amandelbome was ondersoek. Geen van die RBMs het 'n effek op die reproduktiewe knopbreek, vrugset, opbrengs of na-oes kwaliteitsparameters getoon nie. Olie-bevattende behandelings het egter die aanvang van vegetatiewe knopbreek versnel en gelei tot 'n groter oorvleueling in reproduktiewe- en vegetatiewe ontwikkeling. Siende dat geen voor die hand liggende verskille in reproduktiewe ontwikkeling getoon is nie, kan versnelde

vegetatiewe knopbreek moontlike voordele inhou weens die vervroeging in die fotosintetiese vermoë van bome. Olie-bevattende RBMs het ook gelei tot ‘n hoër spoorproduksie in behandelde bome. Siende dat amandel meestal op spore dra, het hierdie behandelings dus die vrugdraende-oppervlakte verhoog en moontlik ook die opbrengspotensiaal vir die daaropvolgende seisoene. Van al die RBMs wat getoets is, was die 5% waterstof-sianamied behandeling, tesame met 2% mineraalolie, die mees effektiefste RBM vir versnelling in vegetatiewe groei en verhoogde vrugdraende-oppervlakte in ‘Independence’ amandelbome verbou onder Suid-Afrikaanse toestande.

Die invloed van kommersiële byekorwe en die teenwoordigheid van ‘n kruisbestuiwer (‘Nonpareil’) op vrugset en kwaliteit is ondersoek. Die teenwoordigheid van hierdie verenigbare kruisbestuiwer het nie ‘n invloed op vrugset, opbrengs of na-oes kwaliteitsparameters getoon nie. Dit dui daarop dat opbrengspotensiaal nie benadeel sal word in die afwesigheid van ‘n kruisbestuiwer in enkelkultivar boorde nie. Die epistigmatiese blomme van ‘Independence’ amandelbome het die kapasiteit vir outogamie, maar dit was nie doeltreffend genoeg om maksimum opbrengste te verseker nie. Die teenwoordigheid van bestuiwers is nodig om suksesvolle selfbestuiwing en –bevrugting te verseker in hierdie selfverenigbare amandelkultivar, indien kommersieël aanvaarbare oesladings verlang word.

NOTE

This thesis is a compilation of chapters, starting with a literature review, followed by three research papers where each paper is an individual entity and some repetition between papers, therefore, has been unavoidable.

TABLE OF CONTENTS

Table of content

GENERAL INTRODUCTION

Almond (*Prunus dulcis* (Mill.) D.A. Webb; synonym *P. amygdalus* Batsch) is genetically similar to other *Prunus* species (Socias i Company, 2017), having a low chill requirement of less than 500 Utah chill units on average (Alonso et al., 2005), accompanied with early flowering and leafing (Socias i Company and Felipe, 1992). These characteristics, in combination with the ability to develop deep and extensive root systems when combined with the right rootstock, gives almond a high tolerance for summer heat and drought (Gradziel et al., 2017), making it ideally suited for production in Mediterranean-type climatic regions, such as the Western Cape. California is the single largest producer of almonds, contributing almost 80% to the global almond supply (California Department of Food and Agriculture, 2019). Despite investigations into the suitability of the Western Cape for almond production dating back to 1980 (Navorsingsinstituut vir Vrugte en Vrugtegnologie, 1985), failure to establish an almond industry has led to local demand substantially outweighing the supply, making South Africa a net importer of almond (Industrial Development Corporation, 2017).

The literature review in this thesis gives a holistic overview of the phenological factors and environmental conditions that need to be considered ensuring successful almond production. Emphasis was placed on dormancy progression, chilling and heat requirements, artificial rest breaking, as well as flowering dynamics in almond.

The mechanism of dormancy enables the phenological adaptation of deciduous fruit trees to their surrounding environments, ensuring survival during unfavourable winter conditions (Cooke et al., 2012; Saure, 1985). Dormancy is regulated by both environmental and inherent factors (Cooke et al., 2012), with chill requirement (CR) and heat requirement (HR) playing a synergistic role in determining dormancy progression and the time of flowering (Luedeling, 2012; Prudencio et al., 2018). Almond, generally, has a low CR to overcome endodormancy (Socias i Company, 2017), as well as a low HR for flowering (Magness & Traub, 1941) compared to other deciduous fruit trees. Paper 1 reports on the progression of dormancy, as well as the CR and HR of ‘Independence’ almond trees grown commercially in the Western Cape.

A lack of winter chilling in deciduous fruit trees can hinder vegetative and reproductive bud break and growth due to incomplete dormancy release, ultimately compromising tree architecture and yield (Erez, 2000). The South African fruit growing regions are characterised

by marginal winter conditions (Linsley-Noakes et al., 1994), therefore, the use of chemical rest breaking agents (RBAs) have become standard commercial practice in both pome and stone fruit production. Chemical RBAs are used to address problems associated with incomplete dormancy release caused by insufficient chill accumulation (Costa et al., 2004; Erez et al., 1971), typically enhancing and advancing bud break. Oil was the first chemical used to artificially break dormancy (Samish, 1945). Mineral oil, in combination with hydrogen cyanamide (HC) is commonly used for rest breaking in deciduous fruit trees (Erez, 1995; Faust et al., 1995), but present risks of phytotoxicity, especially in reproductive buds of stone fruit (Erez, 2000). This can be addressed by chemical treatments with a milder rest breaking effect such as KNO_3, in combination with mineral oil (Erez, 2000; George et al., 2002). The cytokinin-containing growth regulator, thidiazuron (TDZ), have shown promising rest breaking responses in pome and stone fruit (Campoy et al., 2010; Erez et al., 2008; Sagredo, 2008).

Neither of the two largest almond producing countries, namely Australia and the United States of America, constituting more than 85% of global almond production (Australian Almonds, 2020), include chemical rest breaking application as standard almond cultivation practice. Therefore, literature on artificial rest breaking in almond is scarce, while unfavourable climatic conditions have restricted local almond production and expansion in the past. There is thus a need to determine the effect of chemical RBAs on almond trees and evaluate if chemical rest breaking applications hold any benefit to commercial almond production under South African conditions. Paper 2 reports on the efficacy of seven rest breaking treatments in improving bud break and enhancing the effective bearing surface of 'Independence' almond trees cultivated under South African conditions. In relation to this, fruit set and quality was also investigated.

Almond is the only *Prunus* species that is not commercially cultivated for its mesocarp, but instead, for its seed (kernel) (Socias i Company, 2017). Successful pollination and subsequent fertilization are therefore inevitable in almond production. Most of the commercial almond cultivars express self-incompatibility (Socias i Company, 1990) and are therefore reliant on natural pollinators, honeybees in particular, to ensure successful cross-pollination and fertilization among cross-compatible cultivars (Weinbaum et al., 1989; Segura et al., 2017). Natural bee populations are declining globally (Potts et al., 2010), while cultivation of pollinator dependent crops continue to increase (Aizen et al., 2008). The early flowering characteristic of almond further complicates successful cross-pollination due to unfavourable

weather conditions for bee activity (Gradziel et al., 2017; Kester et al., 1996). Breeding for self-compatible almond cultivars could address these problems associated with cross-pollinator dependency, while facilitating various orchard management practices (Socias i Company, 1990). Despite the lack of research done on the true pollinator dependency, many self-compatible almond cultivars, including 'Independence', are labelled as "pollinator independent" (Doll, 2012; Sáez, 2020). Furthermore, self-compatible almond cultivars, express heterozygosity, implying that only half of the pollen grains produced, have the ability to self-pollinate (Dicenta et al., 2002). This raises concern when establishing orchards in the absence of cross-pollinators. There is thus a need for a thorough investigation into pollinator dependence in self-compatible almond cultivars, as well as the effect of cross-pollinators within an orchard. Paper 3 reports on the effect of commercial beehives and cross-pollinator presence on fruit set and quality in 'Independence' almond orchards grown in South Africa. In additions, the quality of flowers from different bearing positions was determined with regards to style and anther morphology.

Literature cited

Aizen, M.A., L.A. Garibaldi, S.A. Cunningham, and A.M. Klein. 2008. Long-term global trends in crop yield and production reveal no current pollination shortage but increasing pollinator dependency. Curr. Biol. 18:1572-1575.

Alonso, J.M., J.M. Ansón, M.T. Espiau, and R. Socias i Company. 2005. Determination of endodormancy break in almond flower buds by a correlation model using the average temperature of different day intervals and its application to the estimation of chill and heat requirements and blooming date. J. Amer. Soc. Hort. Sci. 130(3):308-318.

Australian almonds. 2020. Almond insights 2019-20. Australian Board of Almonds, Loxton, Australia.

California Department of Food and Agriculture. 2019. California agricultural statistics review, 2018-2019. CDFA, CA, USA.

Campoy, J.A., D. Ruiz, and J. Egea. 2010. Effects of shading and thidiazuron + oil treatment on dormancy breaking, blooming and fruit set in apricot in a warm-winter climate. Scientia Hort. 125:203-210.

Cooke, J.E.K., M.E. Eriksson, and O. Junttila. 2012. The dynamic nature of bud dormancy in trees: environmental control and molecular mechanisms. Plant Cell Environ. 35:1707-1728.

Costa, C., P.J.C. Stassen and J. Mudzunga. 2004. Chemical rest breaking agents for the South African pome and stone fruit industry. Acta Hort. 636:295-302.

Dicenta, F., E. Ortega, J.A. Cánovas, and J. Egea. 2002. Self-pollination vs. cross-pollination in almond: pollen tube growth, fruit set and fruit characteristics. Plant Breeding 121:163-167.

Doll, D. 2012. Independence Almond – Some Observations. 7 November 2020. http://thealmonddoctor.com/2012/06/30/independence-almond-some-observations/

Erez, A. 1995. Means to compensate for insufficient chilling to improve bloom and leafing. Acta Hort. 395:81-95.

Erez, A. 2000. Bud dormancy; phenomenon, problems and solutions in the tropics and subtropics, p. 17-48. In A. Erez (ed.). Temperate fruit crops in warm climates. Kluwer Academic Publishers, The Netherlands.

Erez, A., S. Lavee, and R.M. Samish. 1971. Improved methods for breaking rest in the peach and other deciduous fruit species. J. Amer. Soc. Hort. 96:519-522.

Erez, A., Z. Yablowitz, A. Aronovitz, and A. Hadar. 2008. Dormancy breaking chemicals; Efficiency with reduced phytotoxicity. Acta Hort. 772:105-112.

Faust, M., D. Liu, S.Y. Wang, and G.W. Stutte. 1995. Involvement of apical dominance in winter dormancy of apple buds. Acta Hort. 395:47-56.

George, A.P., R.H. Broadley, R.J. Nissen, and G. Ward. 2002. Effects of new rest-breaking chemicals on flowering, shoot production and yield of subtropical tree crops. Acta Hort. 575:835-840.

Gradziel, T.M., R. Curtis and R. Socias i Company. 2017. Production and growing regions, p. 70-86. In: R. Socias i Company and T.M. Gradziel (eds.). Almond: botany, production and uses. CABI, Boston, MA.

Industrial Development Corporation. 2017. Study on the market potential for the commercial viability of almond production in South Africa. OABS Development (Pty) Ltd, Paarl, South Africa.

Kester, D.E., G.C. Martin, and J.M. Labavitch. 1996. Growth and development, p. 90-97. In: W.C. Wicke (ed.). Almond production manual. UCANR Publications, CA, USA.

Linsley-Noakes, G.C., G.W. Matthee, and P. Allen. 1994. Modification of rest completion prediction models for improved accuracy in South African stone fruit orchards. J. S. Afric. Soc. Hort. Sci. 4:13-15.

Luedeling, E. 2012. Climate change impacts on winter chill for temperate fruit and nut production: A review. Sci. Hort. 144:218-229.

Magness, J.R. and H.P. Traub. 1941. Climatic adaptation of fruit and nut crops, p. 400-420. Yearbook of Agriculture. Univ. Press of the Pacific, Honolulu, Hawaii.

Navorsingsinstituut vir Vrugte en Vrugtegnologie. 1985. Inligtingsbulletin nommer 545: Amandelverbouing. NIVV, Stellenbosch, South Africa.

Potts, S.G., J.C. Biesmeijer, C. Kremen, P. Neumann, O. Schweiger, and W.E. Kunin. 2010. Global pollinator declines: trends, impacts and drivers. Trends Ecol. Evol. 25:345-353.

Prudencio, A.S., P. Martínez-Gómez, and F. Dicenta. 2018. Evaluation of breaking dormancy, flowering and productivity of extra-late and ultra-late flowering almond cultivars during cold and warm seasons in South-East of Spain. Sci. Hort. 235:39-46.

Sáez, A., M.A. Aizen, S. Medici, M. Viel, E. Villalobos and P. Negri. 2020 Bees increase crop yield in an alleged pollinator-independent almond variety. Nature Research 10:1-7.

Sagredo, K.X. 2008. Effect of rest-breaking and fruit thinning treatments on reproductive development in apple. Ph.D. dissertation, Univ. of Stellenbosch, Stellenbosch, South Africa. Date las accessed: 10 December 2020. http://library.sun.ac.za/en-za/Pages/Home.aspx

Samish, R.M. 1945. The use of dinitrocresol minreal oil sprays for the control of prolonged rest in apple orchards. J. Pomol. Hort. Sci. 21:164-179.

Saure, M.C. 1985. Dormancy release in deciduous fruit trees. Hort. Rev. 7:239-300.

Segura, J.M.A., R. Socias i Company, and O. Kodad. 2017. Late-blooming in almond: A controversial objective. Scientia Hort. 224:61–67.

Socias i Company, R. 1990. Breeding self-compatible almonds. Plant Breeding Rev. 8:313-338.

Socias i Company, R. 2017. Pollen-style (in)compatibility: development of autogamous cultivars, p. 188-208. In: R. Socias i Company and T.M. Gradziel (eds.). Almond: botany, production and uses. CABI, Boston, MA.

Socias i Company, R. and A.J. Felipe. 1992. Almond: a diverse germplasm. HortScience 27:717–718.

Weinbaum, S.A., D.V. Shaw, and T.T. Muraoka. 1989. Independence of self-compatibility and potentiality for self-pollination in peach × almond hybrids. Euphytica 41:53-58.

LITERATURE REVIEW: Almond production with special reference to dormancy progression, rest breaking and flowering dynamics.

Table of contents

1. Introduction

The almond [*P. dulcis* (Mill.) D.A. Webb; synonym *P. amygdalus* Batsch] is included in the *Rosaceae* family. The almond forms part of the genus *Prunus*, however: it is the only species from this genus grown commercially for its kernel, instead of the mesocarp (Socias i Company, 2017). It is genetically very similar to other stone fruit, but due to the commercial interest in the kernel, it is more often colloquially know as a nut (Socias i Company, 2017). Since the time of its origin in central and south-western Asia (Micke and Kester, 1997) commercial almond production has spread worldwide (Martínez-Gómez et al., 2007). The United States of America (USA), California in particular, is the leading almond supplier worldwide, contributing 79% to the global almond production in 2019. Australia is the second largest almond supplier, producing 7% of the global almond supply, followed by the 6% produced in Spain (Australian Almonds, 2020). In both the USA and Australia, Nonpareil is the leading almond cultivar, followed by Butte and Carmel, respectively (Australian Almonds, 2020; California Department of Food and Agriculture, 2019). Characteristics such as a low chill requirement (CR), early flowering and vegetative growth and high tolerance to summer heat and drought makes almond production ideal for Mediterranean-type climates with mild winter conditions and dry, hot summers (Socias i Company and Felipe, 1992a) such as the Western Cape, South Africa.

Deciduous fruit crops, such as almond, enter a state of dormancy during winter to enable trees to withstand unfavourable climatic conditions and ensures reproduction by delaying the flowering time and fruit set (Campoy et al., 2011a; Faust et al., 1997). Numerous environmental signals, such as lower temperatures and shorter photoperiods, lead to the cessation of growth, ensuring dormancy establishment prior to extreme winter conditions (Allona et al., 2008). Lang et al. (1987) divided dormancy into three stages or phases. The first stage, endodormancy, is controlled by signalling from within the affected structure; the second, ecodormancy, which is controlled by environmental factors; and lastly, paradormancy, where dormancy is induced by signalling from structures other than the effected structure. The relationship between low temperatures and dormancy release (Coville, 1920) led to the concept of CR needed to overcome dormancy (Campoy et al., 2011a). Various chill accumulation models, including the Utah (Richardson et al., 1974), Dynamic (Fishman et al., 1987a, 1987b) and Daily Positive Utah model (Linsley-Noakes et al., 1995) were developed and have since been used to determine the CR of different deciduous fruit cultivars which enabled the estimation of a cultivar's successful adaptation to a pre-determined location (Samish and

Lavee, 1962). However, Campoy et al. (2011a) criticized the lack of understanding of tree physiology in these models, when determining dormancy progression. Besides the CR, Campoy et al. (2011b) found that heat accumulation, together with the accumulation of chill, is necessary for bud development and growth. The heat requirement (HR) concept originated from the argument made by Richardson et al. (1975) that a certain threshold temperature ensures the onset of growth and development after dormancy completion, while temperatures below this base level inhibits growth. The Growing Degree Hour (GDH) model was therefore designed, with a base temperature of 4.5°C and an upper limit of 25°C and used to predict when certain growth and development phases will take place after dormancy release (Richardson et al., 1975). Therefore, the CR and HR of a cultivar play a synergistic role in the survival and reproduction of deciduous fruit trees (Luedeling, 2012).

Incomplete endodormancy release is the result of insufficient winter chill, delaying and/or reducing bud break, while hindering the uniformity of vegetative and reproductive bud break (Erez, 2000). To address the complications associated with incomplete endodormancy release, chemical rest breakings agents (RBAs) have become part of standard cultivation practices in deciduous fruit growing regions with mild winter conditions (Costa et al., 2004; Erez and Lerner, 1990; Sheard et al., 2009), such as South Africa. The yield potential of an almond tree is determined by the survival rate of reproductive buds, dormancy progression and floral development during the subsequent spring (Szalay, 2006). In *Prunus* species, flower buds tend to break prior to vegetative buds (Segura et al., 2017). Saure (1985) has speculated that reproductive buds have a lower chilling requirement, compared to that of vegetative buds, while other studies have indicated a great sink strength expressed in reproductive organs, compared to vegetative organs (Hansen, 1967). Tombesi et al. (2016) indicated that almond yield is less correlated with fruit set percentage and more correlated with the abundance of flowers on the trees. Therefore, better reproductive bud break enhances the possibility of achieving maximum yield potential in almond crop.

Successful pollination and fertilization are essential in producing almond kernels. Commercial almond cultivars are primarily self-incompatible, such as Nonpareil, Butte and Carmel (Socias i Company, 1990; Asai et al., 1996). Compatible cross-pollinator cultivar(s), such as Ne Plus Ultra and Peerles, as well as pollen vectors are needed to ensure successful cross-fertilization, both of which have financial and practical implications. Furthermore, a worldwide increase in cultivation of pollinator dependent crops (Aizen et al., 2008), together with a decline in natural bee populations globally (Potts et al., 2010) increases the demand for

self-compatible cultivars such as Independence and Soletta, which decrease pollinator dependency. The success rate of a self-compatible almond cultivar is determined by the pollen tube growth, fruit set ability and potential to produce a commercially viable crop load following self-pollination (autogamy or geitonogamy), under orchard condition (Socias i Company et al., 2004). Some literature suggests that the spatial ratio between the stigma and anther could serve as an indicator for natural autogamy in some self-compatible almond cultivars, such as Le Grand and various other almond selections (Bernad and Socias i Company, 1995; Weinbaum, 1985). Godini et al. (1992) refuted these findings by showing no correlation between the stigma/anther position and fruit set following hand-pollination of various self-compatible almond cultivars, including Tuano and Genco. However, when comparing fruit set percentage following assisted and unassisted self-pollination, these authors indicated an increase of more than 300% in trees that received hand-pollination. It is therefore concluded that insect vectors are needed to ensure optimal self-pollination in self-compatible almond cultivars, irrespective of stigma/anther position. This highlights the problem that some self-compatible almond cultivars, such as Independence, have been labelled "pollinator independent", even though little research has been done on the true dependency of these cultivars on pollen vectors (Sáez, 2020).

This literature review describes the life cycle of the almond, focusing specially on the progression of bud dormancy, the chilling and heat requirements, rest breaking and the flowering dynamics, including pollination. It aims to discuss the environmental and phenological factors that need to be considered ensuring successful commercial almond cultivation in Mediterranean-type climates characterized by mild winter conditions, such as parts of the Western Cape Province, South Africa.

2. History and background

Almond originated in the mountainous regions and deserts of southwestern and central Asia (Micke and Kester, 1997). Wild almond species have certain characteristics, which led to an interest in scion and rootstock breeding. These traits include kernel composition, early ripening, drought and disease resistance, vigour control and self-compatibility (Socias i Company et al., 2017). The spread of almonds from Greece into Mediterranean coastal regions started around 450 BC and almonds were introduced to the USA by early colonists in the 1700's, however, significant plantings were only made in the mid 1800's in central California (Micke and Kester, 1997).

The Californian almond industry officially began when A.T. Hatch of Suisun, California, selected four seedlings and planted them together, which led to good and consistent cropping. These seedlings were named ‘Nonpareil’, ‘IXL’, ‘Ne Plus Ultra’ and ‘La Prima’. Nonpareil became the industry standard in the marketplace, as well as the orchards with Peerless (selected by Mr Wilson Treat of Colusa) and Ne Plus Ultra serving as the main cross polliniser for these cultivars (Kester et al., 1991). Orchards were planted on hillsides without irrigation, as was the traditional European style, and draught tolerant rootstocks were used (Kester and Ross, 1996). The California almond industry has grown from 14 500 ha bearing trees in 1920 to nearly 400 000 ha in 2013 (Sumner et al., 2014), with almond plantings more than doubling in the last decade. Vast expansions took place during the 1960s and continued to grow until present (Gradziel et al., 2017). In 2017, the USA had 416 800 ha bearing trees, producing more than one million tons of marketable almonds during the 2017/2018 season (United States Department of Agriculture, 2018). Today, California produces more than 70% of the world’s almond supply (Gradziel et al., 2017).

During 1850 – 1900’s, almonds were introduced to other regions with similar climatic conditions to California, such as West Australia, South Africa and parts of South America, notably Argentina and Chile (Kester et al., 1991). In the late 1920’s, the Australian industry took off with their first commercial plantings of 809 ha near Adelaide, with cultivars that were originally from California and Europe, or locally raised seedlings (Quinn, 1941; Wirthensohn and Iannamico, 2017). The almond producing regions of Australia are quite isolated and pollution-free, with abundant solar radiation for photosynthesis and accumulation of kernel nutrients providing a distinct advantage over other nut-producing countries (Wirthensohn and Iannamico, 2017). Second to the USA, Australia is the largest almond producing country in the world, making almond production the largest horticultural export industry in Australia, producing more than 104 000 tons of almond kernels in 2019, contributing 7% to the global almond production (Australian Almonds, 2020).

Spain has been cultivating almonds for more than 2000 years, having more than 500 000 ha of bearing trees in 2012 (Gradziel et al., 2017). Spain was the third largest almond producing country in 2019, contributing 6% (78 089 tons) to the global almond production (Australian Almonds, 2020). The low productivity in Spanish almond orchards has been ascribed to the fact that less than 10% of their almond orchards are irrigated (Gradziel et al., 2017). The two cultivars, Desmayo Largueta and Marcona, are the most widely planted in

Spain and are the only two almond cultivars marketed by name, while the rest of the almond cultivars form part of the generic denomination (Gradziel et al., 2017).

3. South African context

The South African almond industry is very small compared to USA, Australia, Spain, Argentina and Chile. The Mediterranean-type climate of the Western Cape Province, with its mild winters and dry, hot summer conditions, makes this region ideal for almond production. Dr G Kochba from Israel, an expert on almonds, was invited to South Africa in 1980 to investigate the possibility of local almond production. He identified three regions in the Western Cape suited for almond production, namely the Tulbagh and Calitzdorp regions and the Piketberg district, including regions such as Moorreesburg, Malmesbury, Hermon and Gouda (Navorsingsinstituut vir Vrugte en Vrugtegnologie, 1985). An attempt to establish a local almond industry by the South African Dried Fruit Board in the 1970s was halted due to low prices and poor yield (Industrial Development Corporation, 2017). The lack of chill satisfaction and unfavourable spring conditions for adequate cross-pollination for the cultivars available during that time, proved to be limiting factors for the cultivation of almonds under South African conditions (personal communication, Dr P.J.C Stassen). Therefore, the almond industry in South Africa failed to expand in the past due to limited local supply to justify the volume needed to warrant investing in processing facilities (Wirthensohn and Iannamico, 2017).

South Africa is a net importer of almonds, mostly from the USA, with imports valued at R316.34 million for 2 847 tons in 2015. Whereas the South African annual almond production in the past was estimated at 200 to 300 tons (Industrial Development Corporation, 2017), emphasizing the shortage in South African almond supplies to satisfy the local demand. The American company, Zaiger's Inc. Genetics, forming part of the Scientific Research and Development Services Industry, developed the entirely self-compatible almond cultivar, Independence, in 2008. This cultivar originated from a cross between the All-In-One almond cultivar and Almond selection 2168 (Batlle et al., 2017). Since its release, the total number of cultivated hectares under 'Independence' in the USA increased from 16 ha to almost 2000 ha in one decade (California Department of Food and Agriculture, 2019). Likewise, 'Independence' has shown growing popularity with South African growers. In 2016, Zaiger's Inc. Genetic and Zaiger SA granted ZZ2 (Cape Almonds) the exclusive master-licence to cultivate 'Independence' in Southern Africa. Since then, Cape Almonds, in partnership with several sub-licensee growers, have planted more than 600 ha of 'Independence' in various

micro-climates throughout the Western Cape (personal communication, Cape Almonds). Today, the South African almond industry has expanded to more than 1700 ha of various almond cultivars, mostly consisting of Independence and Nonpareil, grown by Cape Almonds, Robertson Almond Company and Unlimited Nuts, in partnership with various growers across the country (personal communication, Robertson Almond Company).

The annual local demand for almond is estimated at 3300 ton, while the local production during the 2019/2020 season was approximately 450 tons, with 350 ton being exported. Produce for the local market is sold as raw almonds to retailers such as Food Lover's Market, as well as processing companies, such as At Source. The value of almonds is comparable to other tree nut crops but has lower production costs compared to other nuts and deciduous fruit. Low production costs, together with relatively simple cultivation practices when compared to other deciduous fruit, have become the driving force for almond plantings in Mediterranean-type regions. Almond production offers a better alternative to marginal crops, such as wine grapes and certain vegetables grown in the Western Cape Province. The lack of large-scale plantings limit the feasibility of processing plants, as well as the cost of harvesting. A lack of knowledge on production under local conditions, together with limited natural resources, particularly water supply, further impedes the expansion of the industry in South Africa (personal communication, Robertson Almond Company).

Even though the South African almond industry has shown an increasing trend over the last four years, it remains a young industry with hardly any documentation of horticultural practices and protocols in place to ensure adequate commercial production of high-quality almond crops. Therefore, studies on the suitability of this crop under South African conditions are needed to determine the viability of commercial almond production.

4. Rootstocks and cultivars

Almond seedlings were primarily used to produce rootstocks due to initial non-irrigated conditions for plantings in Iran, Turkey and other nearby regions (Denisov, 1988; Gradziel, 2009; Gradziel et al., 2001). As production in the USA shifted more to the Central Valley, soils were irrigated and sometimes flooded, therefore, plum and peach rootstocks that are more suitable for these conditions became the preferred option for rootstocks (Gradziel, 2009). In California there are four types of rootstocks generally used, depending on the soil conditions, namely 'Lovell', 'Nemaguard', peach-almond hybrids and 'Mariana' (Micke and Kester, 1997). In the northern parts of California, rain-saturated soils can be problematic for almond

production. Rootstocks such as ‘Marianna’ (plum origin) and ‘Lovell’ (peach origin) are used to address this problem. In the sandy soils of the southern parts of California, nematodes can pose a problem and ‘Nemaguard’ rootstocks would be recommended (Gradziel et al., 2017).

In South Africa, the most common rootstocks used for almond cultivation are of peach-hybrid origin, namely ‘Viking’, ‘Atlas’, ‘GF 677’, ‘Cadaman’, and ‘Flordaguard’ (personal communication, Cape Almonds, Robertson Almond Company). Both ‘Viking’ and ‘Atlas’ rootstocks are interspecific hybrids from *P. persica* x *P. davidiana* x *P. amygdalus* x *P. blireiana*. These rootstocks have desired characteristics such as resistance to root-knot and ring nematodes, tolerant to saline and alkaline soils, as well as high replanting tolerance due to vigorous growth (Dave Wilson Nursery, 2020). ‘GF 677’ rootstocks form part of the *P. persica* x *P. amygdalus* hybrid selection that can establish a well-developed root system, making it suitable for production in infertile soil and drought conditions (Reighard and Loreti, 2008). One of its most renowned features is its suitability to soils high in free-lime (Stassen, 2007). This rootstock is more tolerant to replanting due to high vigour, but not, however, suitable for high-density plantings and exceptionally fertile and heavy soils, due to high sensitivity for root water logging (Reighard and Loreti, 2008). ‘Cadaman’, an interspecific hybrid of *P. persica* x *P. davidiana*, initially induces similar vigour in the scion, compared to ‘GF 677’, with decreasing trends four to five years after orchard establishment. Furthermore, compared to ‘GF 677’, ‘Cadaman’ shows good resistance to water logging, induces earlier precocity and larger fruit size, while maintaining a comparable productivity regarding peach and nectarine production (Reighard and Loreti, 2008). ‘Flordaguard’ is a *P. persica* x *P. davidiana* hybrid that is suitable for production in well-drained sandy soils, due to a high sensitivity for root water logging. Desired traits such as nematode resistance/tolerance, precociousness and low chill requirements make ‘Flordaguard’ suitable for use under South African conditions. Limitations for this rootstock include high vigour and sensitivity to wet and saline conditions (Stassen, 2007).

The contribution of the main almond cultivars planted in California to the total almond production in 2014 is shown in Fig. 1 (Gradziel et al., 2017). The following almond cultivars are registered on the South African fruit varieties list (Department Agriculture, Forestry and Fishery, 2015) of which Nonpareil and Independence are the most widely planted: Britz, Butte, Carmel, El Fahem, Ferragnes, Independence, ‘IXL, Ne Plus Ultra, Nonpareil, Paper Shell, Peerless, Price, Texas Mission and Volcani.

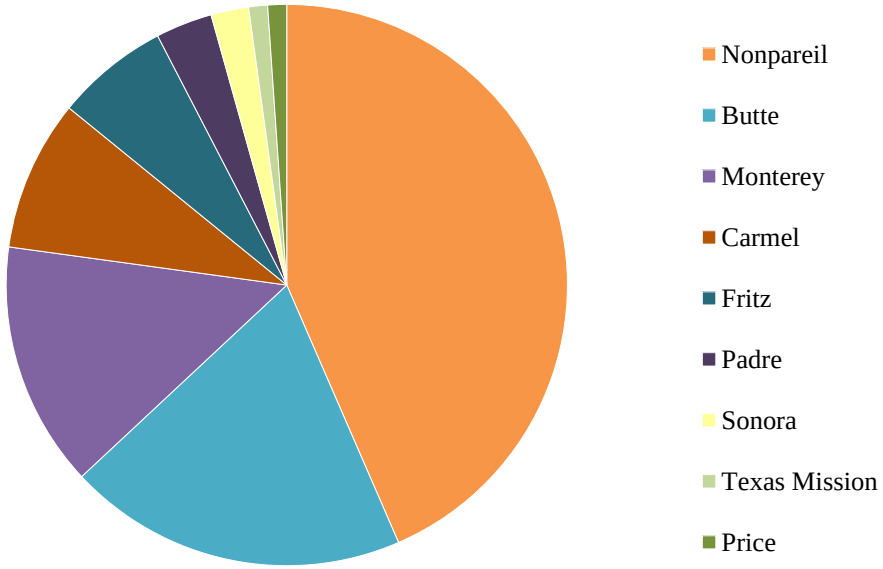


Fig. 1. Contribution of main almond cultivars in California to the total production in 2014 (Adapted from Gradziel et al., 2017).

When establishing an almond orchard, factors such as the growing and environmental conditions, the vegetative growth potential and, especially, the type of machinery used to harvest should be considered (Arquero and Jarvis-Shean, 2017). With this in mind, a between-row width of 7 – 8 m, with an in-row width of 6 – 7 m, is internationally recommended for commercial orchards under irrigation, resulting in 178 to 238 trees per ha (Hendricks, 1996). Casanova-Gascón et al. (2019) have investigated the influence of super-high density (SHD) training systems (2500 trees per ha) on almond production, compared to the conventional open-center training system with 278 trees per ha in Spain. These authors concluded that SHD training systems would be more efficient in almond production, due to a significantly higher yield, however, less productive, due to a lower average almond in-shell weight, compared to open-centre systems. However, the medium- and long-term behaviour of these orchards are not yet known and, therefore, these orchards are still considered at an experimental phase (Arquero and Jarvis-Shean, 2017). For optimal pollination with self-incompatible cultivars, it is recommended that 30% of the orchard should consist of adequate cross-pollinators (Arquero and Jarvis-Shean, 2017). Table 1 comprises specific almond varietal groups that are cross-incompatible due to similar *S*-alleles. Cultivars within a group will not cross-pollinate, while cultivars across groups are compatible cross-pollinators (Asai et al., 1996).

Table 1. Incompatible almond cultivars grouped. Adapted from Asai et al. (1996).

Thompson	Ne Plus Ultra	Nonpareil	Carmel	Solano	Mission	Monterey
Granada	Merced	IXL	Carmel	Eureka	Ballico	Monterey
Harvey	Ne Plus Ultra	Long IXL	Carrion	Kapareil	Languedoc	
Mono	Norman	Nonpareil	Livingston	Solano	Mission	
Robson	Price	Profuse	Monarch	Sonora		
Sauret #2	Ripon	Tardy Nonpareil	Sauret #1	Vesta		
Thompson	Rosetta					
Woods Colony						

To ensure efficient mechanical harvesting, as well as adequate pollination, it is advised that two adjacent rows should be of the self-incompatible cultivar, followed by a cross-pollinator row (Arquero and Jarvis-Shean, 2017).

5. Environmental factors

5.1 Growing season climate

Mediterranean-type regions, such as the Western Cape, South Africa, are characterised by dry, hot summers and mild winter conditions. Almond production is well suited to these climatic conditions, especially when combined with the right rootstock cultivar, ensuring a deep and extensive root system to enhance heat and drought tolerance, combined with less chilling needed for early blooming periods (Gradziel et al., 2017).

Almonds are one of the earliest temperate tree crops to bloom, thus production is limited by the occurrence of frost (Segura et al., 2017). To avoid this risk, almonds were traditionally grown in coastal regions, as crops can be damaged by late winter and early spring frost in higher-lying areas, leading to a marginal crop and causing great economic losses. Despite this, almond production expanded to the inland regions, as well as subtropical regions with lower winter chilling, due to increasing demand (Segura et al., 2017). Late blooming, therefore, became a desirable trait in breeding programs with significant progress being made in the delay of the flowering time to decrease the occurrence of frost incidence in newly released cultivars (Martínez-Gómez et al., 2006). As a consequence, almond cultivars possibly show the widest range of blooming dates among all the fruit and nut species. Unusually cold winters, or the cultivation of almonds in colder regions than what the cultivar is adapted to, could lead to

excessive chilling, causing early flowering and exposing the crop to late spring frost (Segura et al., 2017). Cool temperatures and rain during bloom are also limiting due to interference with cross-pollination and promotion of fungal diseases (Kester et al., 1991).

On the contrary, temperate fruit trees that do not receive adequate winter chilling are prone to incomplete dormancy release, causing delayed and/or low levels of vegetative and reproductive bud break (Erez, 2000). Today, most temperate fruit crops, including almonds, are cultivated in different environmental conditions compared to their place of origin, complicating the process of dormancy breaking in these crops (Egea et al., 2003). This emphasises the need for research regarding the synergism between CR and HR of the different cultivars to ensure effective dormancy release and flowering.

5.2 Dormancy progression and lack of winter chill

Dormancy is an annual developmental phase that enables deciduous fruit trees to survive cold winter conditions in temperate climatic zones (Saure, 1985) and can be defined as "the temporary suspension of visible growth of any plant structure containing a meristem" (Lang et al., 1987). Dormancy breaking was first studied for the use of early forcing of ornamentals and was overlooked by the deciduous fruit industry until the need arose to expand temperate fruit production to less suitable environments with little chill during winter (Saure, 1985). Furthermore Lang et al. (1987) divided dormancy into three classifications types, namely *endodormancy*, *paradormancy*, and *ecodormancy* describing them as follow; *endodormancy* refers to the state where the initial reaction leading to growth is controlled by the recognition of an environmental or endogenous signal within the affected structure (bud) alone, such as chilling and photoperiodic responses. In contrast, if an initiation reaction is caused by a specific biochemical signal that stems from a structure, other than the affected structure, it is said to be *paradormant*, such as apical dominance. Lastly, the state of *ecodormancy*, where buds regain competency to respond to external factors, but remain dormant due to one or more unfavourable environmental factors.

Coville (1920) was the first to indicate the relationship between low temperatures and release from dormancy. Exposure to low temperature for a certain amount of time is necessary to uplift endodormancy in deciduous fruit trees (Linsley-Noakes et al., 1994). Endodormancy has been characterised as a stage that plants enter independently from environmental conditions, although these conditions could promote or inhibit the progression into this stage

(Lang et al., 1987). Plants cannot, however, emerge independently from endodormancy. The duration of this dormant state is inversely proportional to the severity and/or the duration of winter chill (Saure, 1985). Low temperatures during autumn and winter are necessary to break the endodormant state of buds, followed by growth promoting temperatures to overcome ecodormancy and result in flowering during spring (El Yaacoubi et al., 2016). Negation of chill accumulation due to excessively high day temperatures causes inadequate chill accumulation during mild winter conditions and therefore resulting in poor bud break in spring (Erez, 1995). Should bud break occur under these circumstances, vegetative buds form rosettes and reproductive buds do not develop or set normal fruit, especially in stone fruit (Erez, 1999). Erez (1987) described three effects caused by a lack of winter chilling with varying intensities depending on the level of inadequate chilling: "a) poor bud break, poor foliage development, sparse bloom and, frequently, abnormal flowers; b) delayed foliation and bloom and uneven bud break; and c) poor fruit set, reduced leaf area due to lack of growing points, and early growth cessation due to secondary dormancy".

South African deciduous fruit growing regions have moderate to warm winter climates, which negatively affects the dormancy development and release of endodormant buds (Linsley-Noakes et al., 1994). The endodormancy period is likely to be extended in regions with warm winters and needs to be completed before bud break can occur (Saure, 1985). Prolonged dormancy is problematic as it can prevent complete structural development of reproductive buds, resulting in dwarfed pistils, usually leading to abortion of the flower primordia causing abscission of reproductive buds (Black, 1952). In the case of apricots growing in Mediterranean regions, tissue necrosis caused the floral primordia to abort, leading to underdeveloped pistils (Legave et al., 1982). Further studies on apricots found that warm pre-blossom conditions cause a lack in synchronisation between pistil and other floral organ development by accelerating anthesis, opposed to pistil development (Rodrigo and Herrero, 2002), as well as increasing abscission in reproductive buds (Martínez-Gómez et al., 2002). Erez (2000) reported that warm winter conditions may lead to abnormal development of flowers, mostly abnormal ovary development or severe drop of reproductive buds in the case of stone fruit, while the occurrence of bud drop in pome fruit is very rare. An increased rate of defective flowers is to be expected when the winter does not fully satisfy the chilling requirements (Campoy et al., 2010). Conversely, almonds have high summer heat and drought resistance, but continued exposure to high temperatures could lead to the genetic condition known as "non-infectious bud failure" (Kester et al., 1991). This condition has no association with pathogenic organisms but leads to

ultimate tree decline due to failure of vegetative bud development (Fenton et al., 1988), further complicating almond production in regions with mild winter chill. If the reproductive buds of stone fruit do not break in spring, the buds will always die and abscise, compared to those of apples where the reproductive buds may be viable up to a year after staying dormant (George and Erez, 2000). This emphasises the importance of understanding dormancy and rest breaking in deciduous fruit trees. Fig. 2 indicates the breaking of dormancy in floral buds of 'Independence' almond trees, followed by flowering.

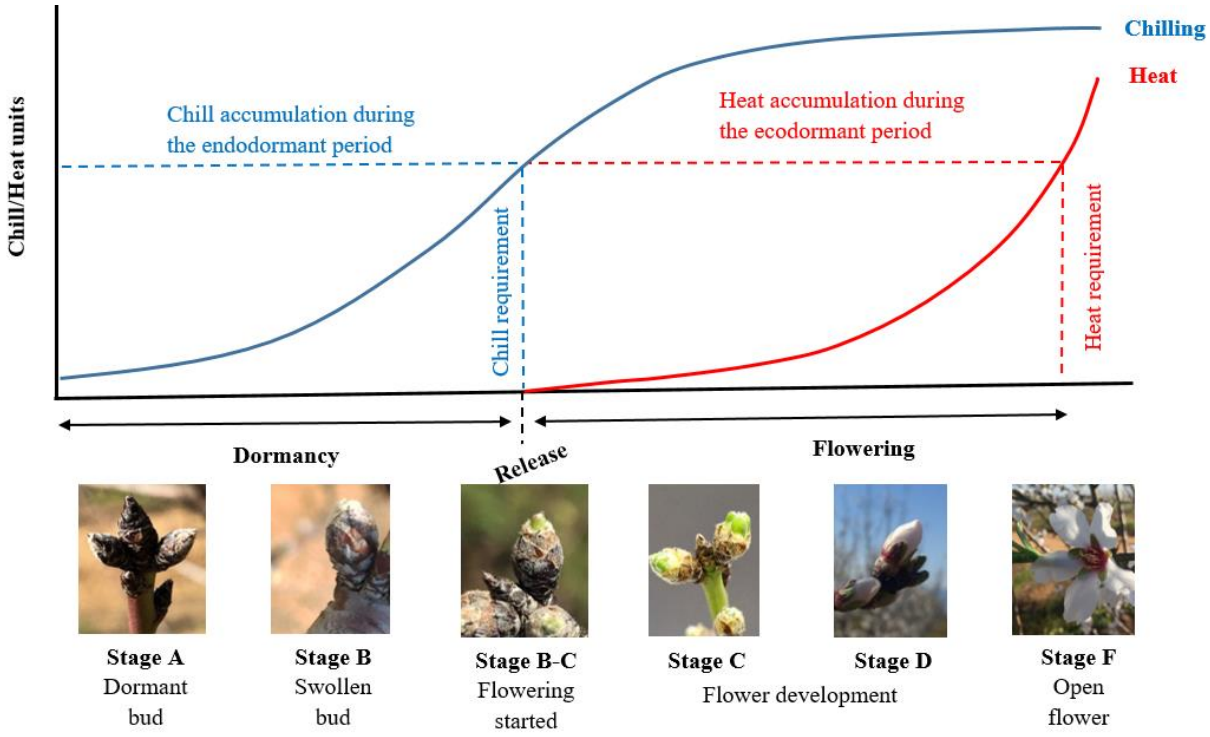


Fig. 2. Dormancy release in reproductive almond buds and subsequent flowering, according to the phenological scale developed by Felipe in 1977 (Adapted from Lang et al., 1987; Luedeling et al., 2009; Prudencio et al., 2018). Photos of 'Independence' flower phenology by T. du Toit.

The survival of deciduous fruit trees during winter depends on the onset of endodormancy prior to extreme temperatures (Campoy et al., 2011a). Environmental signals, such as lower daily minimum temperatures, water stress, light quality and photoperiod causes growth cessation in trees, leading to the establishment of dormancy (Allona et al., 2008). Various studies have been done on the effect of photoperiod on dormancy development in

woody plants, reporting that long days enhance bud break, when compared to natural photoperiod (Erez et al., 1968; Olmsted, 1951; Wareing, 1953). Erez et al. (1968) studied the effect of light on dormancy in peaches and concluded that vegetative bud break was increased by limiting the amount of light supplied during the mid-dormancy period, compared with the natural amount of daylight in winter. Bennet (1950) reported that "light may have a retarding influence in winter and become a stimulating factor in spring and summer". During endodormancy peach trees are affected by fluctuations in light intensity and duration in addition to and apart from temperature (Erez et al., 1968).

5.3 Chill requirement

Chill requirement is a cultivar specific survival mechanism that prevents bud break during autumn, winter and early spring and ensures new growth only takes place during appropriate weather conditions (Lang et al., 1987). Therefore, the CR refers to a minimum period of exposure to low temperatures necessary to overcome dormancy, which protects vulnerable new growth from dying off during winter (Wang, 1960). The CR concept is useful in predicting the ability of a cultivar to adapt to pre-determined environments (Samish and Lavee, 1962). However, due to the high variability in CRs among different cultivars, locations and year, the ability of CR to accurately determine a sufficient amount of cold necessary for a cultivar to overcome endodormancy, came into question (Campoy et al., 2011a).

To address this problem, various linear models have been developed to quantify chilling accumulation of deciduous fruit trees (Gilreath and Buchanan, 1981; Richardson et al., 1974; Shaltout and Unrath, 1983). Richardson et al. (1974) developed a method, called the Utah model, to determine chilling requirement by assigning chill unit (CU) values to different temperature ranges during the endodormancy period. Other adjusted models, regarding the Utah model, soon followed, such as the Low Chilling model (Gilreath and Buchanan, 1981) and the North Carolina model (Shaltout and Unrath, 1983). An important milestone in dormancy modelling was the development of the Dynamic model (Fishman et al., 1987a, 1987b) to address inaccuracies of the Utah model under warm winter conditions. The main adjustment in this model was the fixed chill accumulation by means of a two-step process. Cold temperatures promote the accumulation of an intermediate product, while warm temperatures reverse this accumulation. However, once the accumulated intermediate product reaches a threshold level, Chill Portions (CP) are permanently accrued (Campoy et al., 2011a). The

Dynamic model also demonstrates how the positive effect of cold temperatures can be enhanced by subsequent moderate temperatures between 13°C and 15°C (Erez and Couvillon, 1987; Guerriero et al., 1985). Another improvement, with regards to the Utah model, for warm winter conditions is the Daily Positive Utah model, better locally known as the "Infruitec" model (Linsley-Noakes et al., 1995). The "Infruitec" model accounts for the exposure time to certain temperatures within a cycle, only considering the mechanism of chill negation within a 24-hour period (Linsley-Noakes et al., 1995).

Almonds have a short endodormancy period, as well as a relatively low heat requirement to bring the tree into bloom (Magness & Traub, 1941). Rattigan and Hill (1986) studied twelve different almond cultivars over a seven-year period and found that the CR for these almonds ranged from 220 to 320 CU (CU equation derived from Anderson and Richardson (1982)), while Micke and Kester (1997) indicated that almond CR ranges from 300 to 600 chilling hours below 7°C. Table 2 summarizes the CR and HR of 37 different almond cultivars, foreign and local to Tunisia, from 30 years of phenology records using the Utah, Chilling Hours, Dynamic and Growing Degree Hour model as investigated by Benmoussa et al. (2017). The suitability of the Utah model to determine CR in almond is highly questionable, given the values obtained in this study.

The almond tree is therefore one of the earliest trees to bloom in spring, which makes it extremely susceptible to damage due to moderately late spring frost, limiting production further to regions relatively free from frost (Magness & Traub, 1941). According to Egea et al. (2003), one of the main objectives in breeding programs for temperate fruit is late flowering to avoid winter frost, which can be accomplished by increasing either the chill or post-chill heat requirement of the crop (Segura et al., 2017). Effective bud break temperatures can vary a great deal among different species, and even cultivars within a species (Erez, 1987).

Various environmental factors, such as light intensity, heat, relative humidity, and photoperiod can have an influence on the progression and development of endodormancy in almond, but low temperatures are the single determining factor for its completion (Freeman and Martin, 1981). Therefore, once the chilling requirement of a cultivar is met, endodormancy is concluded. Once endodormancy is completed, reproductive buds progress into an ecodormant state, where growth depends on accumulation of heat (Segura et al., 2017). Once 50% of the reproductive buds in the orchard have reached anthesis, the heat requirement for bud development has been met and ecodormancy is complete. Although there is high variability

among cultivars, almond chill and heat requirements are relatively low and is not currently seen as a limitation to production (Alonso et al., 2005; Segura et al., 2017). However, a difference in CRs among cross-pollinating cultivars may result in a lack in bloom overlap that is necessary to produce a strong yield (Erez, 1987). Due to the relatively low CR of almond, flowering dates in regions characterised by cold winter temperatures seem to be determined by the cultivar's HR, rather than the CR (Alonso et al., 2005; Rattigan and Hill, 1986).

5.4 Heat requirement

In regions with high winter chill, the state of endodormancy in deciduous trees is concluded rather soon, however, bud break might not occur immediately due to unfavourable environmental conditions for growth, causing an imposed ecodormancy. When these conditions turn favourable, rapid growth resumes (Saure, 1985). Therefore, Richardson et al. (1975) argued that temperatures above a certain base level stimulated bud growth and development after endodormancy is completed. These authors further assumed bud growth cessation when temperatures are below this level, while temperatures rising above this level will lead to increased growth. These arguments lead to the development of the Growing Degree Hour (GDH) model having a base temperature of 4.5°C and an upper temperature limit of 25°C. This model was proven successful in predicting the timing of certain growth and developmental phases after endodormancy completion (Richardson et al., 1975). The accumulation of daily mean temperatures above a certain threshold value during the growing season is used to define the plant-temperature relationships and is known as the "heat unit approach" (Wang, 1960).

A synergistic approach between CR and HR has been proposed, that prevents new, vulnerable growth during unfavourable winter conditions, while ensuring growth is initiated early enough for trees to complete their annual reproductive cycle (Luedeling, 2012). Sparks (1993) supports this statement, indicating that both chilling and heating play an interactive role in controlling bud break in pecans; as chill accumulation increases, HR will decrease. According to Sparks (1993) bud break can occur in the absence of chilling, provided that adequate heat is accumulated.

Table 2. The chill and heat requirements of various almond cultivars using the Utah, Chilling Hours, Dynamic and Growing Degree Hour (GDH) model with a base temperature of 4°C. Adapted from Benmoussa et al. (2017).

Cultivar	Utah model (CU)	Chill Hours model (CH)	Dynamic model (CP)	GDH
Abiodh de Sfax	−284	12	4.6	7324
Abiodh Ras Djebel	−53	59	15.5	6206
Achak	−297	8	3.4	8703
Avola	46	50	13.6	6673
Bonifacio	101	61	15.8	7559
Bruantine	−219	34	10.4	8548
Cavaliera	−219	34	10.4	7042
Cristomorto	−29	83	22.6	5872
Dorée	−174	46	12.7	8867
Faggoussi	−148	54	14.5	3962
Fakhfekh	−219	33	10.4	5979
Fasciuneddu	−219	34	10.4	7027
Ferraduel	59	54	14.4	9272
Ferragnes	59	54	14.4	9215
Fournat de Breznaud	−50	79	21.1	5368
Garnghzel	−284	12	4.6	8703
Genco Taronto	194	80	21.4	6148
Khoukhi	−227	31	9.9	8873
Ksontini	−258	21	7.3	7071
Languedoc	−174	23	7.7	9097
Malagueña	−82	23	7.6	9224
Mazzetto	−68	54	14.5	9507
Montrone	−227	31	9.9	9694
Ne Plus Ultra	11	50	13.6	6847
Nonpareil	−29	83	22.6	6045
Pizzuta	−29	83	22.6	2894
Rachelle Taronto	−167	47	13.3	5374
Ramlet R249	18	33	10.3	6812
Ramlet R250	−266	19	6.7	10504
Soukaret	−57	77	20.6	5960
Tarragona	−29	83	22.6	5830
Trell	−57	77	20.6	6003
Tuono Taronto	46	50	13.6	5199
Zahaf	−227	31	9.9	6279

5.5 Rest breaking in deciduous fruit trees

In general, production regions in South Africa accumulate fewer than 1000 Richardson CU per year, resulting in marginal winter conditions for the production of deciduous fruit (Linsley-Noakes, 1994). Insufficient winter chilling in deciduous fruit trees causes delayed foliation, leading to poor bud break, which ultimately compromises the yield and tree architecture. The lack of winter chilling also complicates harvesting and orchard management practices (Costa et al., 2004). To overcome conditions of prolonged dormancy caused by insufficient winter chilling, trials have been done to enhance bud break in peach trees using various substances that are active during dormancy breaking (Luna et al., 1991). Some substances had a greater impact on vegetative buds, while others affected reproductive buds more (Erez et al., 1971). In regions such as South Africa with warm winter conditions, chemical rest breaking agents (RBAs) are successful in synchronising bud break and overcoming problems associated with prolonged dormancy in pome fruit (Costa et al., 2004). Various chemicals are known for their rest-breaking effects. While the active ingredients of these chemicals have little in common, most of them can break dormancy at a sub-lethal dosage (Doorenbos, 1953; Erez, 1987). However, in the absence of chilling, no single chemical RBA can break endodormancy in fully dormant buds, even in low chill cultivars (Erez, 1987).

The nutritional status of a tree and the chemical composition of the rest breaking agent, as well as the rate and timing of application, are the determinant factors of a crop's response to an applied RBA (Erez, 1979; Terblanche and Strydom, 1973). The higher the rate and the later the time of application, the stronger the effect of the treatment, which increases the risk of phytotoxicity. This is especially important in stone fruit which tend to be more sensitive due to the simple composition of their reproductive buds, compared to other fruit such as pome fruit and kiwi which have protected flower buds (Erez, 1987).

Oil served as the first dormancy breaking agent. Plant and animal oil, and later mineral oil, have been used on many deciduous fruit and nut trees, including pistachios, as RBAs to compensate for insufficient chill accumulation (Jarvis-Shean et al., 2015; Sagredo et al., 2005; Samish, 1945). It has been suggested that bud break can be induced by a low oxygen level due to the accumulation of anaerobic end products such as acetaldehyde and ethanol (Erez, 1987; Erez et al., 1980). In the case of mineral oil application, the permeability of oxygen through this oily layer is decreased, causing a gradual decline in oxygen supplied to the enclosed buds (Erez, 1987). Mineral oils have shown promising results in deciduous fruit cultivars with

relatively low CRs, such as Granny Smith apples, but have limited rest-breaking abilities in high chill cultivars such as Golden Delicious (Costa et al., 2004).

Mineral oil was later used in combination with dinitro-o-cresol (DNOC) that acts as an uncoupler of oxidative phosphorylation (Samish, 1945). Oxidative phosphorylation of adenosine diphosphate (ADP) into adenosine triphosphate (ATP) in the mitochondria, is facilitated by electrochemical potential gradient across the mitochondrial membrane (Mitchell, 1961). The impermeability of the inner membrane to protons maintains this proton gradient, while respiration is responsible for its generation (Mitchell, 1961). The DNOC facilitates permeabilization of the mitochondrial membrane (Castilho et al., 1997), thereby inhibiting the mitochondrial ability to maintain ATP synthesis and consequently increasing the rate of respiration rate (Mitchell, 1961; Nicholl, 1982). Temporary anaerobic conditions due to DNOC causes ethanol production though means of fermentation reactions (Møller et al., 2018) which leads to the termination of dormancy and consequent bud break. This reaction is further exacerbated by hypoxic conditions induced by mineral oil (Erez, 1987). To ensure good rest-breaking results, daily temperatures should not be low during application, as well as the following week (North et al., 2012). The effect of this treatment is enhanced by temperatures higher than 24ºC for a few hours, yet it is ineffective if temperatures drop below 12ºC continuously (Erez, 1979). Since the 1940's DNOC, in combination with mineral oil, was the most widely used RBA in deciduous fruit production (Samish, 1945). This treatment combination is relatively low in costs and was a commercially successful RBA on pome fruit in South Africa, but was withdrawn from the market in 2001 due to its extremely toxic traits, both to the environment and humans (Costa et al., 2004; North, 1992; 2003). International markets have pressured deciduous fruit industries to move towards cleaner, more eco-friendly production (Costa et al., 2004).

In Japan, calcium cyanamide ($CaCN_2$) was first used on apples and grapevines as a dormancy breaking agent (Kuroi et al., 1963) and proved successful on raspberries (Snir, 1983) and various other deciduous fruit trees (Morimoto and Kumashiro, 1978). In water, $CaCN_2$ is hydrolysed to soluble calcium hydroxide ($Ca(OH)_2$) and hydrogen cyanamide (H_2CN_2) (Amberger, 2012), the latter acting as the active ingredient during rest breaking. Cyanamide is very reactive and easily taken up by plant tissue, after which it is spread symplastically throughout the plant. Various physiological changes are brought about by cyanamide, of which the key effects are the inhibition of cytochrome oxidase and catalase enzymes (Amberger, 2012). This is similar to the progressive decrease in catalase activity that takes place during

chilling (Patterson et al., 1984). The inhibition of catalase activity by cyanamide reduces the capacity of the cell to scavenge reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), causing cells to be more susceptible to oxidation of membrane lipids (Nir et al., 1986; Shulman et al., 1986). On the other hand, the inhibition of cytochrome oxidase interferes with the electron transport system in the mitochondrial membrane by reducing the electrochemical potential gradient across the membrane. Therefore, inhibition of cytochrome oxidase ultimately reduces adenosine triphosphate (ATP) production, while causing an overall decrease in respiration rate of the plant (Blakenship, 2018). The inability to utilise oxygen induces H_2O_2 production, thereby leading to dormancy breaking in the buds (Vergara et al., 2012). Furthermore, cyanamide increases the polyamine concentration in plant tissue by decarboxylation of storage proteins; polyamines being an essential part of cell division, morphogenesis, flowering, and fruit set (Wang and Faust, 1993).

Hydrogen cyanamide (H_2CN_2) provides comparable rest breaking results to that of DNOC and is less toxic (North, 1992) and thus serves as a suitable alternative for breaking dormancy in various fruit crop (Snir, 1983) such as apples (Costa et al, 2004; Sagredo et al., 2005), peaches and nectarines (Dozier et al., 1990), sweet cherries (Costa et al., 2004; Sheard, 2008), figs (Theron et al., 2011) table grapes (Vergara and Peréz, 2010) and apricots (Bartolini et al., 1997). In severe cases of delayed foliation, cyanamide in combination with mineral oil can enhance the rest breaking abilities (North, 1992; Sagredo et al., 2005; Samish, 1954). However, different fruit genotypes and cultivars differ in correct application time and rate of H_2CN_2 (Fuchigami and Nee, 1987). Late applications delay flowering, while earlier applications have led to more synchronized and advanced bloom in sweet cherries (Snir and Erez, 1988), peaches (Siller-Cepeda et al., 1992) and apples (Bound and Jones, 2004). It is important to note the use of H_2CN_2 has been banned in some countries such as Turkey, due to its carcinogenic effects (İmrak et al., 2016), therefore emphasizing the need to investigate alternative chemical RBAs that can serve as a substitute for H_2CN_2.

Growth regulators, containing plant hormones such as cytokinins and gibberellic acid, have been used to overcome dormancy in deciduous fruit trees (Erez, 1987; Erez et al., 2008; Lloyd and Firth, 1993). Thidiazuron (N-phenyl-N'-1,2,3-thidiazol-5-ylurea) (TDZ) is a cytokinin-like growth regulator that has been effective in breaking dormancy in combination with mineral oil (Campoy et al., 2010; Wang et al., 1986). TDZ stimulates growth in various deciduous crops such as pears, cherries and plums. TDZ in an oil base (in SA trading as Lift®) has been registered for use on apples, with a suggested application rate of 3-4% at 5-6 weeks

before full bloom (Costa et al., 2004). The rate and timing of application depends on the chilling requirement of each cultivars, as well as the chilling received (Costa et al., 2004). Treatments with TDZ seem to increase levels of RNA, DNA, protein, S-adenosylmethionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC), as well as accelerating the formation of polyamine (Wang et al., 1986). It has also been noted that TDZ increases the endogenous cytokinin levels in treated plants (Ferreira et al., 2006). In an experiment done on peaches and nectarines in Israel, Erez et al. (2008) reported that TDZ alone, or in combination with mineral oil, serves as the most powerful chemical RBA they have evaluated. Campoy et al. (2010) indicated that TDZ, in combination with mineral oil, enhanced and advanced flowering and fruit ripening in apricot, however, noted a reduced fruit set percentage under inadequate chill accumulation due to a higher percentage pistil abortion.

According to Erez (1987), potassium nitrate (KNO_3) can also serve as a chemical RBA, while adding two macro-elements to the treated crop. When KNO_3 is applied as a foliar spray, nitrate reductase converts nitrate (NO_3^-) into nitrite (NO_2^-), followed by nitrite reductase converting NO_2^- into nitric oxide (NO) under hypoxic conditions (Shiva, 2012). The reactive nitrogen species (RNS), NO, competes for oxygen, thereby inhibiting cytochrome oxidase (Brown and Borutaite, 2002), while inhibiting catalase activity, causing ROS to accumulate (Brown, 1995). If applied early enough, KNO_3 can be used to address yield problems in certain peach cultivars caused by abnormal flowering, by inducing bud break before abnormal development in these buds occur (Erez, 1987). However, KNO_3 has a mild rest-breaking ability when used alone. Other substances such as Acer® (alkoxylated fatty amine) and Armobreak® (fatty amine polymer) have been used in combination with KNO_3 to enhance its efficacy as a chemical RBA (George et al., 2002; North, 1995). When applying KNO_3 in combination with mineral oil, oxygen deprivation leads to the enzymatic production of NO, thereby causing dormancy breaking in a similar manner to cyanamide (Brown, 1995; Brown and Borutaite, 2002). Stone fruit have simple reproductive buds that are less protected, therefore making them more susceptible to phytotoxicity (Erez, 2000). Due to its mild rest breaking ability (George et al., 2002), KNO_3 could be more suitable for stone fruit to prevent damage to reproductive buds.

Rest breaking treatments can address problems associated with apical dominance (paradormancy) in deciduous fruit trees (Erez, 1987). Faust et al. (1995) indicated that lateral buds enter a much deeper state of dormancy than intact apical buds, however, when apical buds were removed, the CR of the lateral buds was similar to that of the apical bud. Cook and Jacobs (1999) reported that inadequate winter chill hinders acrotonic growth tendencies in apple due

to an enhanced state of paradormant inhibition. Strong apical dominance can therefore be addressed by treating trees with RBA close to terminal bud break, however, a later application time increases the risk of phytotoxicity, especially with chemicals such as cyanamide (Erez, 1987).

6. Plant phenology

6.1 Vegetative growth

In *Prunus* species, vegetative and reproductive buds develop separately, usually with flowering taking place before leafing (Segura et al., 2017). However, Luna et al. (1991) showed the inverse behaviour under laboratory conditions for excised buds from 'Novedad de Córdoba' peach trees that were treated with gibberellic acid or chilling to enhance the dormancy period. These authors went on to study the phenological stages and found that vegetative buds were well-defined after the treatments, while reproductive buds were unresponsive under all conditions. According to their results, the difference in "resting" mechanisms (Hatch and Walker, 1969) of reproductive and vegetative buds were due to differences in the respective tissue development of the buds. Research into the morphological development of the buds showed that floral development was incomplete and ongoing up to a few days prior to the natural flowering period, while vegetative buds were fully developed by midsummer/early autumn (Luna et al., 1991). Therefore, the incidence of vegetative bud break prior to that of reproductive buds under forcing conditions, which is inverse to the general phenomenon of *Prunus* under natural conditions, can be attributed to the continuous morphological development in floral organs. This prevents reproductive buds from breaking in response to increased ambient temperatures, while vegetative bud development is completed prior to forcing conditions and therefore able to start growing.

Rapid vegetative growth takes place after vegetative bud break and leaf production in spring (Doll, 2017). Shoot growth is necessary for the establishment of a sizable canopy during the formative years of an orchard, and at the same time shoot and leaf growth is necessary for the systematic replacement of fruit bearing positions and production of carbohydrate reserves (Doll, 2017; Kester et al., 1996). Kester et al. (1996) described the annual almond shoot growth cycle in five distinct stages that start as soon as buds are released from dormancy and respond to increasing temperatures. These five stages are classified as initiation of growth; shoot elongation; growth cessation; bud dormancy; and endodormancy, marking the end of the

annual growth cycle. Initial growth and development after bud break depends on carbohydrate reserves from the previous season, however, once this early development is completed, photosynthates produced during the current season is responsible for a greater part of the fruit and shoot growth (Hansen, 1971). Studies on 'Big Top' nectarine indicated that newly formed leaves undergo sink-source transition and start to export photosynthetic assimilates 7 – 10 days after bud break, correlating to 32 – 52% of the full leaf expansion (Marchi et al., 2005) Similar results were also found for sour cherry (*P. cerasus* L.) by Kappes and Flore (1989).

6.2 Reproductive growth, flower structure and development

An increase in fruit set of almonds leads to a higher yield, emphasizing the importance of a high flower density on these trees (Ortega et al., 2004). Reproductive buds are borne laterally on both spurs and shoots with vegetative terminal buds ensuring annual elongation (Polito et al., 1996). In shoots longer than 10 cm, reproductive buds tend to be located more towards the terminal end of the shoot, while spurs typically have between one and five flower buds. Inside the reproductive bud is a single, terminal flower without any vegetative structures (Lamp et al., 2001; Polito et al., 1996). Bernad and Socias i Company (1995) indicated that the flowers that open first during bloom were predominantly single and fertile, whilst sterile flowers tend to open later. Higher fertility is predominantly seen in single flowers, receiving better nutrition, therefore developing prior to lower quality flowers (Socias i Company and Felipe, 1994). Flower quality can be attributed to pistil development and the resulting ability to set fruit, however, various other factors such as flower size, stigma receptivity and ovule longevity can influence the quality of a flower (Williams, 1965). Ovule development is correlated to ovary width in almonds (Socias i Company et al., 1976), therefore flower sterility or fertility is morphologically indicated by the width of the ovary.

There are three determining factors for the timing of bloom in almonds, namely the amount of chill accumulation during winter, the subsequent exposure to warm temperatures after winter and before bloom, and the threshold temperature for bud growth (Polito et al., 1996). Rattigan and Hill (1987) has described flowering in almonds as a two-stage process, the first being bud dormancy release when the chilling requirement is met, while during the second stage the rate of bud development is controlled by a base level temperature, in this case 4.5°C. Sufficient chill accumulation leads to endodormancy completion, followed by the ecodormant state of bud development during which heat accumulates, leading to anthesis and thereby

marking the end of ecodormancy (Segura et al., 2017). Lamp et al. (2001), describes almond flower bud differentiation in eight phases. During the first phase, Stage 0, the apical meristem is in the vegetative state, producing bud scales before flower initiation occurs. The enlargement of the meristem at the shoot apex indicates the start of flower initiation, representing Stage 1, after which the apex broadens and thickens, forming an elongated, broad dome. Bract primordia are produced on the apex periphery by the dome during Stage 2. The single, terminal flower differentiating at the floral apex is subtended by typically three of these bracts. Stage 3 is characterized by the initiation of five sepals at the periphery of the terminal apex, indicating the start of organogenesis. Alternate to the sepals, petal primordia arise within the calyx during Stage 4. During Stage 5 sequential stamen initiation takes place within the corolla. Stage 6 is characterized by growth and development of the calyx, corolla and stamen bases forming the hypanthium, while the floral apex becomes concave. During the final phase, Stage 7, a single primordium emerges at the meristem periphery, indicating carpel initiation, and expands along the flanks of the apical meristem. The margins of the carpel develop, forming a single carpel that completely covers meristem apex (Lamp et al., 2001).

As previously mentioned, Luna et al. (1991) found that reproductive peach buds were not responsive to forcing conditions, even after gibberellic acid (GA_3) or chill treatments. Further research by these authors indicated that development in vegetative buds were complete during midsummer/early autumn, while these buds remained dormant until the end of winter. During this period, verticils in the reproductive buds differentiated continuously, leading to the bloom of sterile flowers in January (July; southern hemisphere (SH)). Mid-February marks the active transition (initiation) of an almond apical meristem into a flower, followed by the development of microscopic flower parts along its flanks during March (SH), April (SH) and part of May (SH) (Polito et al., 1996). Sepal and petal development in reproductive buds takes place during late summer, while development of the androecium occurred throughout the winter, joined by the differentiation of the gynoecium during late winter (Luna et al., 1991). Polito et al. (1996) found that pollen develops from the male reproductive cells during late May to early June (SH), with the pollen grains present by mid-June (SH). Furthermore, pistil initiation starts early in April (SH) and development continues until early June (SH), with little further development in the reproductive structure until early July (SH), when two ovules are formed within the locule. Luna et al. (1991) also found that differentiation of the reproductive buds was completed only a few days prior to natural flowering. The lack of responsiveness to treatments in reproductive buds in this study was therefore attributed to the insufficient

development of verticils, in particular the andoecium and gynoecium, preventing sprouting. Reinoso et al. (2002) supports these findings after suggesting "cell division, enlargement, and differentiation, which lead to organogenesis, take place throughout the entire 'dormancy' period" in reproductive peach buds. A visual representation of the phenological development in reproductive buds of 'Independence' almond trees is presented in Fig. 3.

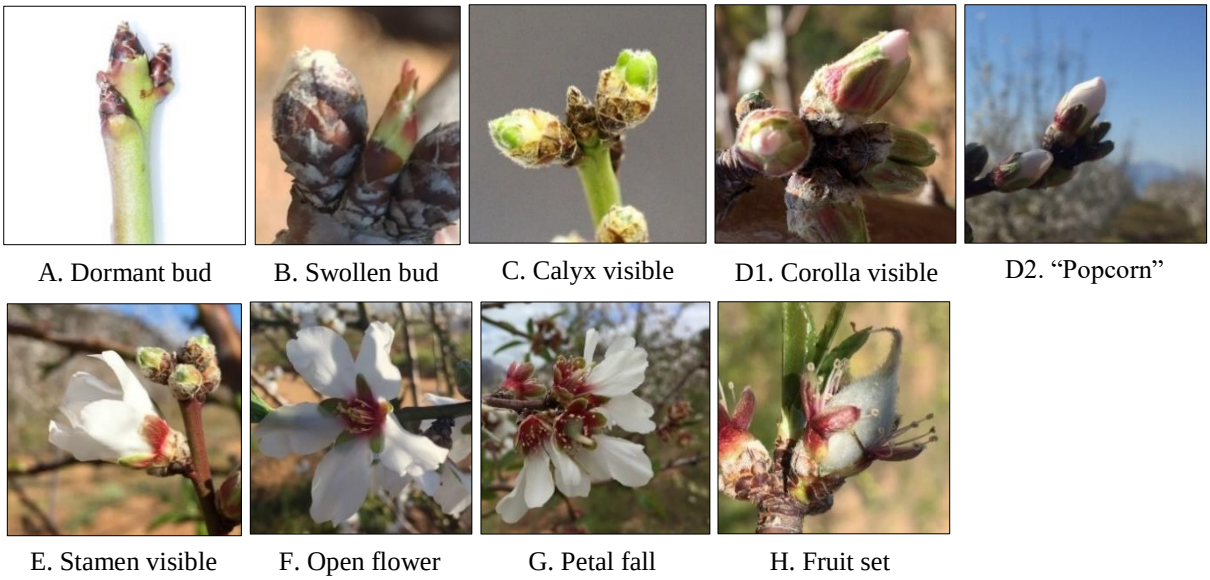


Fig. 3. The stages of phenological development in 'Independence' floral buds according to the scale developed by Felipe in 1977. Adapted from Sánchez-Pérez et al. (2012) and Thomas and Connell (2018). Photos by T du Toit.

In most plant species with natural populations, flowering time is subject to genetic variation, which leads to different adaptations to various climatic conditions (Segura et al., 2017). Kester et al. (1973) and Dicenta et al. (1993) described late flowering in almonds as a polygenetic trait. However, a number of other studies on late blooming almond cultivars have led to the discovery of a single dominant gene, *Lb* (late bloom), that could be involved in determining the date of flowering (Kester, 1965; Jones, 1972; Socias i Company et al., 1999). According to Socias i Company et al. (1999), the presence of this allele indicates that the date of flowering is a quantitative, as well as a qualitative trait, with late flowering dominant over early flowering. Almond cultivars with higher reproductive bud densities would be preferred in regions that have problems related to frost, such as Spain (Kodad and Socias i Company, 2008), due to the correlation between abundance of flowers and almond yield potential

(Tombesi et al., 2016). This might also explain the high reproductive bud density in some peach cultivars from regions such as Hungary, Canada and USA (Werner et al., 1988; Okkie and Werner, 1996; Szabó et al., 1998). However, it has been noted that floral quality can become problematic in almond cultivars with high flower densities due to competition among flowers (Socias i Company and Felipe, 1994), which is why cultivars with low/medium reproductive bud densities are preferred in regions such as California, where late frost is not as problematic as in Spain.

Almond trees tend to produce a considerably higher number of flowers compared to the fruit that set, yet it is important that practically all flowers are effectively pollinated. An increase in the set of the remaining flowers cannot compensate for inadequate pollination or the loss of a portion of the flowers (Griggs and Iwakiri, 1964). Likewise, thinning actions are not required in almond production, because a reduced kernel size is not a marketing disadvantage (Polito et al., 1996). According to Tombesi et al. (2016), almond tree yield seems to be correlated more with the abundance of flowers on a tree than the relative number of fruit that set.

6.3 Pollination requirements

The early flowering characteristic of almonds often makes them susceptible to unfavourable weather conditions during bloom, which can interfere with pollination (Bernad and Socias i Company, 1995). Polito et al., (1996) described pollination as transfer of pollen from the anther to the stigma, while fertilization is defined as the union of the male germ cell embedded in the pollen grain with the female germ cell (the egg) contained in the ovule. The ovules grow and develop from July until bloom, depending on the temperature (Polito et al., 1996). Successful fertilization is therefore an inevitable prerequisite for almond crop production. Within a carpel, two ovules, a primary and secondary ovule, usually differentiate and develop in *Prunus* species. After pollination, the secondary ovule generally aborts, which leaves the primary ovule subject to fertilization, forming a single kernel (Pimienta and Polito, 1982). Double-kernelled fruit develop when both ovules remain viable (Polito et al., 1996), which is not commercially valued due to deformation of the kernels (Egea and Buros, 2000).

Pollination and the subsequent growth of the pollen tube down the style of the flower must occur rapidly to ensure that a viable ovule is fertilized (Ortega et al., 2004). The receptivity of the ovule was first assessed by Williams (1965) by of determining the effective

pollination period (EPP) of the flower. This concept has since been described as the period during which effective fertilization of flowers takes place and can be determined by the longevity of the ovule minus the number of days needed for pollen tube growth. Several conditions influence the receptivity of a stigma to pollen and thus the duration of the EPP, including environmental, physiologic and genetic conditions (Ortega et al., 2004). Griggs and Iwakiri (1964) were the first to investigate the criticality of timing for effective cross-pollination in 'Nonpareil' almond flowers. In their studies, results showed that under weather conditions favourable for natural cross-pollination, the highest percentage fruit set was found in flowers cross-pollinated one to two days after opening. Flowers remained receptive for three to four days after opening, but had a significantly lower fruit set. They also found that cross-pollination one to two days before the flowers were completely open lead to a significantly lower fruit set, indicating the importance of maturity in almond pistil receptivity to pollen germination and fertilization. A later study also by Griggs and Iwakiri (1975) found that it still took four to five days for pollen tubes to grow and reach the ovary in 'Nonpareil' and 'Texas' ('Mission') flowers. Earlier pollination of flowers after opening can thus increase the chances of fertilization and adequate fruit set. Early pollination and rapid growth of pollen tubes are critical to ensure a viable ovule is reached in almond cultivars with ovules that are already mature during anthesis (Ortega et al., 2004).

Various factors affect pollination and fertilization of almond flowers. However, the most limiting factor for fruit set is rain during the flowering period, due to the enhanced risk for floral diseases and the suppression of cross- and self-pollination (Gradziel and Weinbaum, 1999), while high relative humidity limits anther dehiscence (Corbet, 1990). A few hours after the flowers open, anther dehiscence begins, which leads to the shedding of pollen. Optimum temperatures for anther dehiscence ranges from 18 – 26ºC, while temperatures lower than or equal to 15ºC can retard dehiscence (Polito et al., 1996). Once the pollen is transferred from the anther to the stigma of a receptive pistil, pollen germination can take place (Taiz et al., 2018) with optimal temperatures ranging from 10 - 21ºC for rapid pollen germination (Polito et al., 1996). Under favourable weather conditions, the stigma is receptive as soon as the flower opens and up to 3 or 4 days thereafter. Cool, cloudy, or wet weather extends the receptive period, while temperatures higher than 27°C, low humidity and wind shortens this period (Polito et al., 1996). Henselek et al. (2018) indicated that between 80 and 100 pollen grains need to be deposited on the stigma to ensure successful fertilization in almond. After pollination, the pollen tube grows from the stigma, down the style to the ovule (Taiz et al.,

2018). Ambient temperatures above 25ºC can cause a decline in pollen tube growth, while tubes are likely to burst at temperatures exceeding 32ºC. Temperatures below 15ºC can also lead to a decline in the rate of pollen tube growth but can be compensated for by a decrease in the declining rate of the ovule (Polito et al., 1996). The growth of the pollen tube through the style of an almond flower can take as long as three to four days under orchard conditions, with an additional two to four days for the pollen tube to reach an ovule (Polito et al., 1996).

Different insect species, such as syrphid flies and ants, are effective pollinators, yet the only species that is commercially viable and readily available is the European honeybee (*Apis mellifera* Linnaes) (Le Feuvre, 2017). Under field conditions, honeybees serve as the primary pollen transfer between cultivars. According to Thorp (1996), wind contributes very little to pollination of almond flowers. The obstruction of bee visitation will therefore result in a failure to produce a commercially acceptable crop load (Le Feuvre, 2017; Micke and Kester, 1997).

Pollen-foraging bees are the most efficient pollinators for almond flowers, compared to nectar-foraging bees that have minimal contact with anthers and stigma (Thorp, 1996). Henselek et al. (2018) found that the miner bee (*Andrena cerasifolli* Cockerell) was more effective in pollinating almond flowers when counting the number of flowers with pollen tubes that reached the ovary from a single visit, compared to the honeybee. However, honeybees spent less time on each flower per visit, indicating a higher efficiency compared to the other pollinators. Environmental conditions influence the foraging activity of honeybees, with foraging taking place at temperatures greater than or equal to 12ºC. Honeybees do not forage during rain or strong winds, while cloudiness reduces their flight activity (Thorp, 1996). Many variables influence the number of colonies recommended per hectare of almond trees, including weather conditions during bloom, competing plants, colony strength, as well as the number of bees foraging (Thorp, 1996). For self-incompatible almond cultivars, 3.5 – 10 hives per hectare is recommended (Micke and Kester, 1997). Even though Godini et al. (1992) found that self-compatible almond cultivars could reach optimal fruit set (25 – 40%) (Kester and Griggs, 1959) after natural and artificial pollination, their results indicated that optimum self-pollination can only be ensured in the presence of adequate insect vectors.

The recognition of the importance of bees for pollination in the USA has led to an increase in the demand, while the American almond industry almost doubled in the last decade (Lee et al., 2018). Honeybee populations have been declining globally (Pettis and Delaplane, 2010; Potts et al., 2010) due to agricultural intensification that has led to habitat loss of wild

pollinators (Biesmeijer et al., 2006; Watanabe, 1994). Crop fields planted in isolation from natural habitats further decreases the rate of pollinator visits, leading to a decrease in fruit set (Garibaldi et al., 2011; Kremen et al., 2002; Winfree et al., 2009). Aizen et al. (2008) emphasised the increasing demand for commercial pollinator services in both developed and developing countries, due to a significant increase in the cultivation of pollinator dependent crop, compared to non-dependent crop. The growing dependency on pollinators increases the financial implication of producing crop such as almond.

6.4 Self-incompatible vs self-compatible

Gametophytic self-incompatibility is expressed in most *Prunus* species (Tao and Iezzoni, 2010; Yamane and Tao, 2009). The self-incompatibility locus, *S*, is responsible for the recognition and rejection of self-pollen during sexual reproduction in plants (Socias i Company, 1990). Proteins expressed by alleles in either the male or female reproductive organs determine whether the pollen grains will be accepted or rejected by the stigma during pollination. For self-incompatible species, pollen is rejected when the stigmatic cells and pollen grains carry the same *S*-alleles, while being accepted when the alleles differ, allowing pollination and subsequent fertilization (Taiz et al., 2018). Commercial almond cultivars are predominantly self-incompatible, with a few exceptions (Socias i Company, 1990). The display of self-incompatibility in almonds is the result of a lower pollen grain retention due to the stigma rejecting incompatible male gametes; pollen germination being delayed and reduced; lower frequencies of pollen tubes growing down the style; and a delay in the development of the embryo sac (Pimienta and Polito, 1983; Pimienta et al., 1983). A need for compatible cross-pollinators to ensure successful pollination and fertilization in self-incompatible almond cultivars have been established in the early 1900's, which greatly improved the production of almond crops (Micke and Kester, 1997). Due to the common phenomenon of self-incompatible, almond trees depend on honeybees for pollen transport (Weinbaum et al., 1989) among compatible cross-pollinator cultivars during overlapping flowering times (Segura et al., 2017).

Due to the early flowering characteristics of almond (Segura et al., 2017), weather conditions are generally challenging for honeybee pollination (Bernad and Socias i Company, 1995). Therefore, later flowering cultivars that bloom in more favourable conditions, as well as reducing the pollen dependency through means of self-compatibility, have become some of the main focus points in almond breeding (Bernad and Socias i Company, 1995; Socias i

Company, 1990). The development of self-compatible almond cultivars has also been emphasised to address management problems in orchards, particularly during harvest, with two or more cultivars (Socias i Company, 1990). Since the early 1970s, research has been done on identifying almond self-compatibility (Socias i Company, 2017). A successful self-compatible almond cultivar is characterised by similar pollen tube growth, fruit set and commercial crop load following self-pollination (autogamy or geitogamy), compared to that achieved after cross-pollination with compatible pollen (allogamy), under orchard condition (Socias i Company et al., 2004).

Pollination conditions for self-compatible almonds may differ from conditions required in self-incompatible cultivars, but some environmental conditions, such as extreme temperatures and frost, pose a potential threat to both. Ambient temperatures affect the actual time and duration of flowering (Richardson et al., 1975; Samish, 1954; Swartz and Powell, 1981) which could have implications regarding the successful cross-pollination of self-incompatible cultivars if flowering times do not overlap. Some authors have argued that the possibility of natural self-pollination is determined by the position of the stigma relative to the anthers and plays an important role in the quality of self-compatible almond flowers (Bernad and Scoias i Company, 1995; Weinbaum, 1985). In contrast to this, Godini et al. (1992) found no correlation between fruit set and the reciprocal stigma/anther position within a flower, following unassisted self-pollination in eight self-compatible almond cultivars. Results, however, showed that high levels of self-pollination cannot be assured by self-compatible almond genotypes without assistance. This emphasizes the importance of pollinating insects in ensuring successful pollination and fertilization in commercial almond orchards, even if cultivars are entirely self-compatible (Godini et al., 1992; Socias i Company and Felipe, 1992b). However, research in the past has predominantly focused on self-pollination versus assisted pollination (i.e. hand-pollination) (Godini et al., 1992, 1994; Vargas et al., 1997; Weinbaum, 1985) while limited published studies are available on the true dependency of self-compatible almond cultivars on natural pollinators (Sáez et al., 2020).

6.5 Fruit set, nut development and composition

Fruit set is one of the most important factors in determining a genotype's productive potential and the commercial value of the cultivar (Socias i Company et al., 1998). Various factors limit fruit set, including insufficient pollination and/or poor metabolic resource availability that impair continued fruit development (Stephenson, 1981). A reduced number of flowers or conditions leading to less success in flower pollination will ultimately lead to

reduced fruit set and yield (Polito et al., 1996). As discussed earlier, the onset of flowering is particularly important for fruit set, due to higher quality flowers usually being the first to open (Socias i Company, 1983). After successful pollination of the flower and fertilization of the ovule, fruit development can commence (Taiz et al., 2018). Rapid fruit growth takes place during March and April (September and October (SH)), while development of the edible part of the almond fruit, the cotyledon (kernel), is accelerated during April and May (October and November, (SH)) and ceases during mid-June (mid-December, (SH)) (Martínez-Gómez et al., 2008). These authors indicated that the growth rate of the cotyledon is related to the date of flowering and ripening and, therefore, cultivar dependent. The gradual hardening of the endocarp (shell) progresses until fruit ripening (Gradziel and Martínez-Gómez, 2002).

During the ripening phase of almond development, photosynthates start to accumulate in the embryo and continues until maturity, for as long as vascular connections are maintained. The kernel receives carbohydrates by means of transport through the phloem, while the xylem provides transport for the minerals and nitrogen (Kester et al. 1996). Most of the carbohydrates are converted into lipids and amino acids, which will ultimately form proteins, while some carbohydrates remain unchanged (Kester et al. 1996). To ensure full kernel size and optimal weight, healthy foliage and sufficient water availability must be maintained (Kester et al., 1996).

Fruit maturity is indicated when the mesocarp starts to desiccate and split (hull-split), while an abscission layer forms where the nut is connected to the peduncle (Kester et al., 1996). Both the occurrences are influences by the internal moisture content of the tree (Kester et al., 1996) and therefore irrigation practices (Doll and Shackel, 2015). Trees subject to moisture stress could cause “stick-tight” nuts, where hulls tend to tighten on the shell instead of normal hull splitting, whereas too much water can delay the onset of harvest due to prolonged hull split duration (Doll and Shackel, 2015; Kester et al., 1996). The period after hull split and before harvesting is critical for proper irrigation management, as kernel weight and quality can be compromised due to deficit irrigation (Doll, 2017).

7. Harvesting and processing

An optimal time for harvesting is indicated by a relatively dry dehiscent hull. Depending on the cultivar, the metabolism of the almond tree starts to slow down at 25 – 28 weeks after full bloom, initiating the start of senescence (Carbó and Connell, 2017). Auxin levels start to decrease, while the ethylene and abscisic acid activities increase in the peduncle.

Enzymes that hydrolyse polysaccharides in the cell wall, are synthesised, which leads to cell separation and the formation of an abscission layer at the cementation layer of the peduncle, spreading to bordering tissue until the xylem vessels are reached, ultimately leading to nut drop (Taiz, 2018). At the same time, the hull starts to dehydrate, splitting along the suture line (Carbó and Connell, 2017).

Almond trees are usually harvested commercially for the first time during the fourth growing season. Harvesting starts late in February and continues until early in April (SH), while early-ripening cultivars are harvested from mid-January (SH) (Connell et al., 1996). Manual harvesting is not feasible for almonds due to labour constraints, therefore, harvesting takes place mechanically in most orchards due to the lower production costs compared to manual labour costs, while saving time and effort (Carbó and Connell, 2017). Carbó and Connell (2017) describes the harvest process as follow: the first step in almond harvesting is removing the nuts from the trees by means of a mechanical shaker. Various shakers are available, however, the multidirectional shaker is most effective and removes up to 95% of nuts under optimal conditions, while harvesting 50 to 60 trees per hour. After trees have been shaken, nuts are left on the orchard floor to dry for five to ten days, depending on the weather conditions. After the nuts have reached an optimal moisture content, 6% in the case of local processing prerequisite, mechanical sweepers blow the nuts from under the trees and sweep them together in the centre of the rows. This is followed by a mechanical pickup operation, where a pickup machine collects the almonds and reloads them into a conveyer nut cart attached to the back of the pickup machine. Inside the nut cart, foreign material such as leaves, small rocks and twigs are separated from the nuts and left behind in the orchard, while nuts are transported to processing plants where they will be hulled and shelled.

Hulling machines need to be carefully adjusted to ensure optimal kernel quality, as equipment adjusted for the removal of stick tights lower the total output, while more sensitive adjustments will lead to a higher percentage stick tights in the output (Freeman and Connell, 1996). After the almonds have been hulled, the in-shell product is passed through a cracking machine to separate the kernels form the shell. During the following screening, foreign objects such as twigs, shells, stones, etc. is removed, as well as any kernels with severe defects. The almond kernels are then classified according to size using a calibration machine (Verdú et al., 2017).

8. Post-harvest aspects and quality parameters

The quality of almonds depends on four factors as described by Kader (1996). Firstly, the appearance of the product. High quality in-shell products should not have stains or adhering pieces of hull attached, while shelled products are free of insect damage, shrivelling and discolouration, while defective kernels, such as broken and chipped, doubles and rotten/mouldy kernels are removed. The second factor determining the quality, is the texture of the kernels, with optimal ranging between a degree of crisp and chewy. The moisture content determines the texture of the kernels, with moisture contents of 4% and lower resulting in a lower quality product. The third factor described, is the flavour and nutrient composition of the kernels. Taste properties such as the desired level of sweetness and oiliness characterise high quality kernels, while rancidity and staleness is absent. The nutrient content and chemical composition of almonds in general, as directed by the United States Department of Agriculture (USDA), are summarised in Table 3. The final quality factor is the absence of contamination such as microbiological contamination and aflatoxin, which is mainly produced by the fungi *Aspergillus parasiticus* and *A. flavus*, leading to substances that are carcinogenic in the almond kernels (Kader, 1996).

Table 3. The standard composition of natural, whole almonds per edible portion (100 g). Adapted from California Almonds (2020).

Proximate		Minerals		Vitamins	
Energy	575 Kcal	Potassium	705.0 mg	Vitamin E	26.2 mg
Lipids	49.4 g	Phosphorus	484.0 mg	Niacin	3.4 mg
Saturated fatty acids	3.7 g	Magnesium	268.0 mg	Riboflavin (B_2)	1.0 mg
Monounsaturated fatty acids	30.9 g	Calcium	264.0 mg	Pantothenic Acid	0.5 mg
Polyunsaturated fatty acids	12.1 g	Iron	3.7 mg	Thiamin (B_1)	0.2 mg
Proteins	21.2 g	Zinc	3.1 mg	Vitamin B_6	0.1 mg
Dietary fiber	12.2 g	Manganese	2.3 mg	Folate	50.0 mcg
Cholesterol	0.0 g	Sodium	1.0 mg		

Almonds are sold as both in-shell and shelled products, both of which grading is defined by the United States Department of Agriculture (USDA). California is the leading almond supplier globally and have set the international standard for post-harvest quality and grading. South African almond produce is therefore also graded according to USDA standards (personal

communication, Robertson Almond Company). For in-shell almonds, products from different season's crops are not allowed. A moisture content of maximum 6.5% is acceptable in the northern hemisphere (NH) from August, 15th until February, 28th, after which the maximum moisture content allowed is 6.25%. Furthermore, the hulls need to be completely removed (Verdú et al., 2017). Table 4 summarizes the USDA grading system for in-shell almonds, starting with the in-shell product with the highest quality requirements (California Almonds, 2015).

Table 4. The USDA grades for in-shell almonds. Adapted from California Almonds (2015).

USDA grades	Thickness (mm)	External defects	Dissimilar	Under size	Foreign material	Internal (kernel) defects
U.S. No. 1 (Supreme)	11.1	10%	5%	5%	2%	10%
U.S. No. 1 mixed	11.1	10%	-	5%	2%	10%
U.S. No. 2	11.1	10%*	5%	5%	2%	10%
U.S. No. 2 mixed	11.1	10%*	-	5%	2%	10%

*Kernel quality for U.S. No. 2 grade meets requirement of U.S. No. 1, with an additional 20% of shell damage and discolouration allowed.

For the shelled almond product, food legislation requirements with regards to food quality and safety need to be adhered to, such as the presence of aflatoxins, pesticide residue, microbial contamination, product traceability, etc. As in the case of the in-shell product, a maximum moisture content of 6.75% is acceptable for shelled almonds in the NH from August, 15th until February, 28th, after which the maximum moisture content allowed is 6.5%. No foreign materials are allowed, such as pieces of shells, hulls, twigs, stones, etc. (Verdú et al., 2017). Table 5 summarizes the USDA grading system for shelled almonds, starting with the shelled product with the highest quality requirements, U.S. Fancy, and U.S. Standard sheller runner with the least strict quality requirements for whole kernels (California Almonds, 2015).

Table 5. The USDA grades for shelled almonds, with x indicating no limit. Adapted from California Almonds (2015).

USDA grades	Whole kernel	Minimum diameter (mm)	Dis-similar	Doubles	Chip and scratch	Foreign material	Particles and dust	Split and broken	Other defects	Serious defects	Under size

U.S. Fancy	-	-	5%	3%	5%	0.05%	0.1%	1%	2%	1.0%	-
U.S. Extra No. 1	-	-	5%	5%	5%	0.05%	0.1%	1%	4%	1.5%	-
U.S. No. 1 (Supreme)	-	-	5%	15%	10%	0.05%	0.1%	1%	5%	1.5%	-
U.S. Select sheller run	-	-	5%	15%	20%	0.10%	0.1%	5%	3%	2.0%	-
U.S. Standard sheller run	-	-	5%	25%	35%	0.20%	0.1%	15%	3%	2.0%	-
U.S. No.1 whole and broken	30%	8	5%	35%	x	0.20%	0.1%	x	5%	3.0%	5%
U.S. No. 1 pieces	x	3.2	x	x	x	0.20%	1.0%	x	5%	3.0%	5%

Nonpareil is the dominant commercial almond cultivar with the widest range of market uses due to its aesthetic features such as uniform, fairly flat, and light coloured (blonde) kernels, as well as a preferred taste profile (Gradziel et al., 2017). Practical advantages such as consistently high yield and a high shelling percentage (65 – 70%) due to its paper shell, further explains why Nonpareil is the most produced cultivar globally (Asai et al., 1996) and has therefore become the industry standard used to compare other cultivar traits (personal communication, Robertson Almond Company).

9. Conclusion

Inquiries into the suitability of the Western Cape for commercial almond production dates back to the 1980's (Navorsingsinstituut vir Vrugte en Vrugtegnologie, 1985), yet the almond industry in South Africa is currently still in its infancy. An increasing trend in new plantings in the past five years occurred, however, no scientific research has been done on the suitability of almond cultivation under South African conditions, despite failure to expand in the past, partly due to environmental limitations. Almond breeding programmes have been successful in producing later flowering (Martínez-Gómez et al., 2006), low-chill cultivars to address such climatic issues hindering successful pollination and fertilization. Independence is a newly released self-compatible almond cultivar that aims to address problems associated with

unsuitable climatic conditions for almond production in the past, as well as declining rates of natural pollinators experienced today (Batlle et al., 2017; Bernad and Socias i Company, 1995; Pettis and Delaplane, 2010; Potts et al., 2010).

Deciduous trees use the mechanism of dormancy to phenologically adapt to their surrounding environment, thus, affecting the production potential (Campoy et al., 2011a; Cooke et al., 2012). The progression of bud dormancy during winter plays a key role in annual plant development, as it determines the quality of bud break and development in reproductive and vegetative organs. A complex interaction between intrinsic and environmental factors regulate dormancy progression (Cooke et al., 2012). A better understanding of the annual development and environmental responses of a plant during different growth stages could, therefore, aid in determining the successful adaptation to a particular environment, as well as overcome possible limitations in such an environment. This emphasizes the importance of dormancy research for deciduous fruit cultivation, especially in temperate growing regions. Additionally, CR and HR are important adaptive traits specific to each cultivar (Egea et al., 2003; Campoy et al., 2011a), determining the time of dormancy progression and flowering (Prudencio et al., 2018). Investigation of CR and HR, together with dormancy progression, is therefore necessary to estimate a cultivar's suitability within a certain production region.

Inadequate chill accumulation is a major limiting factor for deciduous crop production due to the adverse effect of incomplete dormancy release on reproductive and vegetative growth and development (Erez, 2000). The use of chemical RBAs to address these problems, have become standard practice for pome and stone fruit production in South Africa, due to marginal winter conditions (Linsley-Noakes et al., 1994). In the past, commercial almond cultivars have failed to adapt to local environmental conditions, limiting production and the development of an established almond industry in South Africa (Industrial Development Corporation, 2017). Consequently, detailed studies on chemical RBA uses in almond is necessary to elucidate whether this cultivation practice is necessary for commercial almond production under South African conditions, given the limited attention it has received in literature.

Due to the global increase in cultivation of pollinator dependent crop, accompanied by a current declining rate of natural pollinator populations (Aizen et al., 2008; Lee et al., 2018; Pettis and Delaplane, 2010; Potts et al., 2010), self-compatible cultivars have become a priority in almond breeding programmes (Socias i Company, 1990). Substantial research has been done

on the autogamic ability of self-compatible almond cultivars, with regards to their flower morphology (Bernad and Socias i Company, 1995; De Palma and Godini, 1994; Godini et al., 1992, 1994; Kumar and Kumar, 2000; Vargas et al., 1997; Weinbaum, 1985). Most results have indicated that in commercial orchards, self-compatibility alone is not sufficient to ensure a commercially acceptable crop load, especially in the absence of pollinating insects (Godini et al., 1992; Socias i Company and Felipe, 1992b). In spite of these findings, Sáez et al. (2020) stated that many self-compatible almond cultivars are currently advertised as "self-fertile" and "pollinator independent" without thoroughly establishing the true dependency on pollen vectors. Therefore, intensive research is needed depicting the true dependency of these self-compatible cultivars on natural and supplemented pollinator populations, as this has practical and financial implications for almond production.

10. Literature cited

Aizen, M.A., L.A. Garibaldi, S.A. Cunningham, and A.M. Klein. 2008. Long-term global trends in crop yield and production reveal no current pollination shortage but increasing pollinator dependency. Curr. Biol. 18:1572-1575.

Allona, I., A. Ramos, C. Ibanez, A. Contreras, R. Casado, and C. Aragocillo. 2008. Molecular control of winter dormancy establishment in trees. Span. J. Agric. 6:201-210.

Alonso, J.M., J.M. Ansón, M.T. Espiau, and R. Socias i Company. 2005. Determination of endodormancy break in almond flower buds by a correlation model using the average temperature of different day intervals and its application to the estimation of chill and heat requirements and blooming date. J. Amer. Soc. Hort. Sci. 130(3):308–318.

Anderson, J.L. and E.A. Richardson, 1982. Utilizing meterological data for modelling crop and weed growth, p. 449-461. In: J.L. Hatfield and I.J. Thomason (eds.). Biometeorology in Integrated Pest Management. Academic Press, NY, USA.

Arquero, O. and K. Jarvis-Shean. 2017. Orchard management, p. 240-243. In: R. Socias i Company and T.M. Gradziel (eds.). Almond: botany, production and uses. CABI, Boston, MA.

Asai, W.K., W.C. Micke, D.E. Kester, and D. Rough. 1996. The evaluation and selection of current varieties, p. 52-61. In: W.C. Wicke (ed.). Almond production manual. UCANR Publications, CA, USA.

Australian Almonds. 2020. Almond insights 2019-20. Australian Board of Almonds, Loxton, Australia. Date accessed: 2 December 2020 https://2q1ee4456oc52trll42uctl1-wpengine.netdna-ssl.com/wp-content/uploads/2020/07/2020_Almond_Insights.pdf

Bartolini, S., C. Vitagliano, F. Cinelli, and G. Scalabrelli. 1997. Effect of hydrogen cyanamide on apricot bud break and catalase activity. Acta Hort. 441:159-166.

Batlle, I., F. Dicenta, R. Socias i Company, T.M. Gradziel, M. Wirthensohn, and F.J. Vargas. 2017. Classical genetics and breeding, p. 111-148. In: R. Socias i Company and T.M. Gradziel (eds.). Almonds: botany, production and uses. CABI, Boston, MA.

Bennet, L.J. 1950. Temperature and bud rest period. Effect of temperature and exposure on the rest period of deciduous plant leaf buds investigated. Calif. Agr. 4:11-16.

Benmoussa, H., M. Ghrab, M.B. Mimoun, and E. Luedeling. 2017. Chilling and heat requirements for local and foreign almond (*Prunus dulcis* Mill.) cultivars in a warm Mediterranean location based on 30 years of phenology records. Agr. For. Meteorol. 239:34-46.

Bernad, D. and R. Socias i Company. 1995. Characterization of some self-compatible almonds. II. Flower phenology and morphology. HortScience 30:321-324.

Biesmeijer, J.C., S.P. Roberts, M. Reemer, R. Ohlemüller, M. Edwards, T. Peeters, A.P. Schaffers, S.G. Potts, R. Kleukers, C.D. Thomas and J. Settele. 2006. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. Science 313:351-354.

Black, M.W. 1952. The problem of prolonged rest in deciduous fruit trees. In Proc. 13th Intern. Hort. Congr. 2:1122-1131.

Blankenship, R.E. 2018. Photosynthesis: The light reactions, p. 171-202. In: L. Taiz, E. Zeiger, I.A. Møller, and A. Murphy (eds.). Plant physiology and development. International sixth addition. Oxford Univ. Press, NY, USA.

Bound, S.A. and K.M. Jones. 2004. Hydrogen cyanamide impacts on flowering, crop load, and fruit quality of red 'Fuji' apple (*Malus domestica*). N. Z. J. Crop Hort. Sci. 32:227-234.

Brown C. 1995. Reversible binding and inhibition of catalase by nitric oxide. Eur. J. Biochem. 232:188-191.

Brown C. and V. Borutaite. 2002. Nitric oxide inhibition of mitochondrial respiration and its role in cell death. Free Radic. Biol. Med. 33(11):1440-1450.

California Almonds. 2015. California almonds technical information. Almond Board of California, CA, USA. Date last accessed: 29 November 2020. https://www.almonds.com/sites/default/files/2020-04/abc_technical_kit_2015_0.pdf

California Department of Food and Agriculture. 2019. 2018 California almond acreage report. CDFA, Sacramento, CA, USA. Date last accessed: 29 November 2020. https://www.nass.usda.gov/Statistics_by_State/California/Publications/Specialty_and_Other_Releases/Almond/Acreage/201904almac.pdf

California Almonds. 2020. Guide to California almonds. Almond Board of California, CA, USA. Date last accessed: 29 November 2020. https://www.almonds.com/sites/default/files/content/attachments/guide_to_california_almonds_technical_poster.pdf

Campoy, J.A., D. Ruiz, and J. Egea. 2010. Effects of shading and thidiazuron + oil treatment on dormancy breaking, blooming and fruit set in apricot in a warm-winter climate. Scientia Hort. 125:203-210.

Campoy, J.A., D. Ruiz, and J. Egea. 2011a. Dormancy in temperate fruit trees in a global warming context: A review. Scientia Hort. 130:357-372.

Campoy, J.A., D. Ruiz, N. Cook, L. Allderman, and J. Egea. 2011b. High temperatures and time to budbreak in low chill apricot ‘Palsteyn’. Towards a better understanding of chill and heat requirements fulfilment. Scientia Hort. 129:649-655.

Cape Almonds. 2017. Prospectus: Independence Cultivar. ZZ2, Mooketsi, South Africa.

Carbó, J.L.E and J.H. Connell. 2017. Almond harvesting, p. 406-427. In: R. Socias i Company and T.M. Gradziel (eds.). Almond: botany, production and uses. CABI, Boston, MA.

Casanova-Gascón, J., M, Figueras-Panillo, I. Iglesias-Castellarnau and P. Martín-Ramos. 2019. Comparison of SHD and open-center training systems in almond tree orchards cv. 'Soleta'. Agronomy 9:1-15.

Castilho, R.F., J.A.F. Vincente, A.J. Kowaltowski, and A.E. Vercesi. 1997. 4,6-Dinitro-*o*-cresol uncouples oxidative phosphorylation and indices membrane permeability transition in rat liver mitochondria. Int. J. Biochem. Cell Biol. 29:1005-1011.

Connell, J.H., G.S. Sibbett, J.M. Labavitch, and M.W. Freeman. 1996. Preparing for harvest, p. 254-259. In: W.C. Wicke (ed.). Almond production manual. UCANR Publications, CA, USA.

Cook, N.C. and G. Jacobs. 1999. Suboptimal winter chilling impedes development of acrotony in apple shoots. HortScience 34(7):1213-1216.

Cooke, J.E.K., M.E. Eriksson, and O. Junttila. 2012. The dynamic nature of bud dormancy in trees: environmental control and molecular mechanisms. Plant Cell Environ. 35:1707-1728.

Corbet. S.A. 1990. Pollination and the weather. Israel J. Bot. 39:13–30.

Costa, C., P.J.C. Stassen and J. Mudzunga. 2004. Chemical rest breaking agents for the South African pome and stone fruit industry. Acta Hort. 636:295-302.

Coville, F.V. 1920. The influence of cold in stimulating the growth of plants. J. Agric. Res. 20:151-160.

Dave Wilson Nursery, 2020. Orchard trees: Rootstock comparison. CA, USA. Date last accessed: 11 December 2020. https://www.davewilson.com/product-information-general/rootstock/comparisons

Denisov, V.P. 1988. Almond genetic resources in the USSR and their use in production and breeding. Acta Hort. 224:299-306.

De Palma, L. and A. Godini. 1994. Stigma/anther spatial relationship in self-fertile and self-sterile almonds. Acta Hort. 373:161-165.

Department Agriculture, Forestry and Fishery. 2015. South African variety list as maintained by the registrar of plant improvement. DAFF, Pretoria, South Africa.

Dicenta, F., J.E. García, and E.A. Carbonel. 1993. Heritability of flowering, productivity and maturity in almond. J. Hort. Sci. 68:113-120.

Doll, D. 2017. Almond water requirements, p. 279-291. In: R. Socias i Company and T.M. Gradziel (eds.). Almond: botany, production and uses. CABI, Boston, MA.

Doll, D. and K. Shackel. 2015. Drought management for California almonds. Univ. of California ANR Publication 8515, CA, USA. Date last accessed: 10 December 2020. https://doi.org/10.3733/ucanr.8515

Doorenbos, J. 1953. Review of literature on dormancy in buds of woody plants. Meded. Landbhoogsch. Wageningen. 53:1-23.

Dozier, W.A., A.A. Powell, A.W. Caylor, N.R. McDaniel, E.L. Carden, and J.A. McGuire. 1990. Hydrogen cyanamide induces bud break of peaches and nectarines following inadequate chilling. HortScience 25:1573-1575.

Egea, J. and L. Burgos. 2000. Ovule differences between single-kernelled and double-kernelled fruits in almond (*Prunus dulcis*). Ann. appl. Biol. 136:291-295.

Egea, J., E. Ortega, P. Martínez-Gómez, and F. Dicenta. 2003. Chilling and heat requirements of almond cultivars for flowering. Environ. Exp. Bot. 50:79–85.

El Yaacoubi, A., G. Malagi, A. Oukabli, I. Citadin, M. Hafidi, M. Bonhomme, and J. Legave. 2016. Differentiated dynamics of bud dormancy and growth in temperate fruit trees relating to bud phenology adaptation, the case of apple and almond trees. Int. J. Biometeorol. 1695–1710.

Erez, A. 1979. The effect of temperature on the activity of oil + dinitro-o-creso1 sprays to break the rest of apple buds. HortScience 14:141-142.

Erez, A. 1987. Chemical control of bud break. HortScience 22:1240-1241.

Erez, A. 1995. Means to compensate for insufficient chilling to improve bloom and leafing. Acta Hort. 395:81-95.

Erez, A., 1999. Dormancy completion - A dual response. HortScience 34:524-525.

Erez, A. 2000. Bud dormancy; phenomenon, problems and solutions in the tropics and subtropics, p. 17-48. In A. Erez (ed.). Temperate fruit crops in warm climates. Kluwer Academic Publishers, The Netherlands.

Erez, A. and G.A. Couvillon. 1987. Characterization of the influence of moderate temperatures on rest completion in peach. J. Amer. Soc. Hort. Sci. 112:677-680.

Erez, A. and H. Lerner. 1990. Means to improve leafing using rest-avoidance technique in peaches in Israel. Acta Hort. 279:239-246.

Erez, A., S. Lavee, and R.M. Samish. 1968. The effect of limitation in light during the rest period on leaf bud break of the peach (*Prunus persica*). Physiol. Plant. 21:759-764.

Erez, A., S. Lavee, and R.M. Samish. 1971. Improved methods for breaking rest in the peach and other deciduous fruit species. J. Amer. Soc. Hort. 96:519-522.

Erez, A., G.A. Couvillin, and S.J. Kays. 1980. The effect of oxygen concentration on the release of peach leaf buds from rest. HortScience 15:39-41.

Erez, A., Z. Yablowitz, A. Aronovitz and A. Hadar. 2008. Dormancy breaking chemicals; efficiency with reduced phytotoxicity. Acta Hort. 772:105-112.

Faust, M., D. Liu, S.Y. Wang, and G.W. Stutte. 1995. Involvement of apical dominance in winter dormancy of apple buds. Acta Hort. 395:47-56.

Faust, M., A. Erez, L.J. Rowland, S.Y. Wang, and H.A. Norman. 1997. Bud dormancy in perennial fruit trees: physiological basis for dormancy induction, maintenance, and release. HortScience 32:623–629.

Fenton, C.A.L., A.H. Kuniyuki and D.E. Kester. 1988. Search for a viroid etiology for noninfectious bud failure in almond. HortScience. 23:1050-1053.

Ferreira, W.M., G.B. Kerbauy, J.E. Krause, R. Pescador, and R.M. Suzuki. 2006. Thidiazuron influences the endogenous levels of cytokinins and IAA during the flowering of isolated shoots of Dendrobium. J. Plant Physiol. 163:1126-1134.

Fishman, S., A. Erez., and G.A. Couvillon. 1987a. The temperature-dependence of dormancy breaking in plants – mathematical analysis of a 2-step model involving a cooperative transition. J. Theor. Biol. 124:473-483.

Fishman, S., A. Erez, and G.A. Couvillon. 1987b. The temperature-dependence of dormancy breaking in plants – computer stimulation of processes studied under controlled temperatures. J. Theor. Biol. 126:309-321.

Fuchigami, L.H. and C.C. Nee. 1987. Degree growth stage model and rest-breaking mechanisms in temperate woody perennials. HortScience 22:836-845.

Freeman, M.W. and G.C. Martin. 1981. Peach floral bud break and abscisic acid content as affected by mist, light and temperature during rest. J. Amer. Soc. Hort. Sci. 106:333–336.

Freeman, M.W. and J.H. Connell. 1996. Preventing tree injury during shaking, p. 265-267. In: W.C. Wicke (ed.). Almond production manual. UCANR Publications, CA, USA.

Garibaldi, L.A., I. Steffan-Dewenter, C. Kremen, J.M. Morales, R. Bommarco, S.A. Cunningham, L.G. Carvalheiro, N.P. Chacoff, J.H. Dudenhöffer, S.S. Greenleaf and A. Holzschuh. 2011. Stability of pollination services decreases with isolation from natural areas despite honey bee visits. Ecol. Lett. 14:1062-1072.

George, A.P. and A. Erez. 2000. Stone fruit species under warm subtropical and tropical climates. In A. Erez (ed.). Temperate fruit crops in warm climates. Kluwer Academic Publishers, Dordrecht, Netherlands.

George, A.P., R.H. Broadley, R.J. Nissen, and G. Ward. 2002. Effects of new rest-breaking chemicals on flowering, shoot production and yield of subtropical tree crops. Acta Hort. 575:835-840.

Gilreath, P.R. and D.W. Buchanan. 1981. Rest prediction model for low-chilling ‘Sungold’ nectarine. J. Amer. Soc. Hort. Sci. 106:426-429.

Godini, A., L. de Palma, and M. Palasciano. 1992. Role of self-pollination and reciprocal stigma/anthers position on fruit set in eight self-compatible almonds. HortScience 27:887-889.

Godini, A., L. de Palma, and M. Palasciano. 1994. Self-fertile almonds and fruit set by optimized self- and cross-pollination. Acta Hort. 373:157—160.

Gradziel, T.M. 2009. Almond (*Prunus dulcis*) Breeding, p. 1-31. In: S. Mohan Jain and P.M. Priyadarshan (eds.). Breeding plantation tree crops: Temperate species. Springer, NY, USA.

Gradziel, T.M. and S.A. Weinbaum. 1999. High relative humidity reduces anther dehiscence in apricot, peach, and almond. HortScience. 34(2):322-325.

Gradziel, T.M. and P. Martínez-Gómez. 2002. Shell seal breakdown in almond is associated with site of secondary ovule abortion. J. Amer. Soc. Hort. Sci. 127:69-74.

Gradziel, T.M., P. Martínez-Gómez, and A.M. Dandekar. 2001. The use of S-allele specific PCR analysis to improve breeding efficiency for self-fertility in almond. HortScience 36:440-440.

Gradziel, T.M., R. Curtis and R. Socias i Company. 2017. Production and growing regions, p. 70-86. In: R. Socias i Company and T.M. Gradziel (eds.). Almond: botany, production and uses. CABI, Boston, MA.

Griggs, W.H. and B.T. Iwakiri. 1964. Timing is critical for effective cross pollination of almond flowers. Calif. Agr. 18:6–7.

Griggs, W.H. and B.T. Iwakiri. 1975. Pollen tube growth in almond flowers. Calif. Agr. 29(7):4–6.

Guirriero, R., S.E.P. Indiogine, and G. Scalabrelli. 1985. The effect of cyclic and constant temperatures in fulfilling the chilling requirements of two apricot cultivars. Acta Hort. 192:41-48.

Hansen, P. 1967. ^{14}C-Studies on Apple Trees. I. The effect of the fruit on the translocation and distribution of photosynthates. Physiol. Plant. 20:382-391.

Hansen, P. 1971. ^{14}C-Studies on Apple Trees. VII. The early seasonal growth in leaves, flowers and shoots as dependent upon current photosynthates and existing reserves. Physiol. Plant. 25:469-473.

Hatch, A.H. and D.R. Walker. 1969. Rest intensity of dormant peach and apricot leaf buds as influenced by temperature, cold hardiness and respiration. J. Amer. Soc. Hort. Sci. 94:304-307.

Hendricks, L.C. 1996. Orchard planning, design, and development, p. 47-60. In: W.C. Wicke (ed.). Almond production manual. UCANR Publications, CA, USA.

Henselek, Y., E.J. Eilers, C. Kremen, S.D. Hendrix, and A.M. Klein. 2018. Pollination requirements of almond (*Prunus dulcis*): Combining laboratory and field experiments. J. Econ. Entomol. XX:1-8.

İmrak, B., A.B. Küden, A. Küden, A.K. Sarıer, and B. Çimen. 2016. Chemical applications affected dormancy breaking in 'Modi' apple cultivar under subtropical conditions. Acta Sci. Pol. Hortoru. 15:265-277.

Industrial Development Corporation. 2017. Study on the market potential for the commercial viability of almond production in South Africa. OABS Development (Pty) Ltd, Paarl, South Africa.

Jarvis-Shean, K., D. Da Silva, N. Willits, and T.M. De Jong. 2015. Using non-parametric regression to model dormancy requirements in almonds. Acta Hort. 1068:133–140.

Jones, R.W. 1972. Titan, a seed source for F1 almond × Nemaguard peach hybrids. Fruit Var. Hort. Dig. 26:18–20.

Kader, A.A. 1996. In-Plant Storage, p. 274-277. In: W.C. Wicke (ed.). Almond production manual. UCANR Publications, CA, USA.

Kappes, E.M. and J.A. Flore. 1989. Phyllotaxy and stage of leaf and fruit development influence initiation and direction of carbohydrate export from sour cherry leaves. J. Amer. Soc. Hort. Sci. 114:642-648.

Kester, D.E. 1965. Inheritance of time of bloom in certain progenies of almond. Proc. Amer. Soc. Hort. Sci. 87:214–221.

Kester, D.E. and W.H. Griggs. 1959. Fruit setting in the almond: the effect of cross-pollinating various percentages of flowers. Proc. Amer. Soc. Hort. Sci. 74:206-213.

Kester, D.E. and N.W. Ross. 1996. History, p. 1-2. In: W.C. Wicke (ed.). Almond production manual. UCANR Publications, CA, USA.

Kester, D.E., P. Raddi, and R. Asay. 1973. Correlation among chilling requirements for germination, blooming and leafing in almond. Genetics 74:135-135.

Kester, D.E., T.M. Gradziel, and C. Grasselly. 1991. Almonds (*Prunus*). Genetic resources of temperate fruit and nut crops. 290:701-760.

Kester, D.E., G.C. Martin, and J.M. Labavitch. 1996. Growth and development, p. 90-97. In: W.C. Wicke (ed.). Almond production manual. UCANR Publications, CA, USA.

Kodad, O. and R. Socias i Company. 2008. Significance of flower bud density for cultivar evaluation in almond. HortScience 43:1753–1758.

Kremen, C., N.M. Williams and R.W. Thorp. 2002. Crop pollination from native bees at risk from agricultural intensification. Proc. Natl. Acad. Sci. USA. 99:16812-16816.

Kumar, K. and U. Kumar. 2000. Flower morphology versus self-compatibility in almond. Acta Hort. 522:97-102.

Kuroi, I., Y. Shiraishi, and S. Imano. 1963. Studies on breaking the dormancy of grapevines. I. Effects of lime nitrogen treatment for shortening the rest period of glasshouse grown grapevine. J. Japan. Soc. Hort. Sci. 32:175-180.

Lamp, B.M., J.H. Connell, R.A. Duncan, M. Viveros, and V.S. Polito. 2001. Almond flower development: Floral initiation and organogenesis. J. Amer. Soc. Hort. Sci. 126(6):689–696.

Lang, G.A., J.D. Early, G.C. Martin, and R.L. Darnell. 1987. Endo-, para-, and ecodormancy: Physiological terminology and classification for dormancy research. HortScience 22:371-377.

Lee, H., D.A. Sumner, and A. Champetier. 2018. Pollination markets and the coupled futures of almonds and honey bees: Simulating impacts of shifts in demands and costs. Amer. J. Agr. Econ. 101:230-249.

Le Feuvre, D. 2017. Honeybee pollination services for the Australian almond industry. Australasian Agribusiness Perspective 20(11):194–205. Date last accessed: 10 December 2020. https://cpb-ap-se2.wpmucdn.com/blog.une.edu.au/dist/f/1377/files/2018/09/AAP-Vol-21-Paper-11-Le-Feuvre-162j8du.pdf

Legave J.M., G. Garcia and F. Marco. 1982. Some descriptive aspects of drops process of flower buds, or young flowers observed on apricot tree in South of France. Acta Hort. 121:75-82.

Linsley-Noakes, G.C. 1994. Chapter 3: Development of a rest-completion prediction model for Western Cape climatic conditions, p. 40-68. Ph.D. dissertation, Univ. of Natal, Durban, South Africa.

Linsley-Noakes, G.C., G.W. Matthee, and P. Allen. 1994. Modification of rest completion prediction models for improved accuracy in South African stone fruit orchards. J. S. Afric. Soc. Hort. Sci. 4:13-15.

Linsley-Noakes, G.C., M. Louw, and P. Allen. 1995. Estimating Daily Positive Utah Chill Units using daily minimum and maximum temperatures. J. S. Afr. Soc. Hort. Sci. 5:19-23.

Lloyd, J. and D.J. Firth. 1993. Effect of hydrogen cyanamide and promalin on flowering, fruit set and harvest time of 'Flordaprince' peach (*Prunus persica* (L.) Batsch) in subtropical Australia. J. Hort. Sci. 68:177-183.

Luedeling, E. 2012. Climate change impacts on winter chill for temperate fruit and nut production: A review. Sci. Hortic. 144:218-229.

Luedeling, E., M. Zhang, and E.H. Girvetz. 2009. Climatic changes leading to declining winter chill for fruit and nut trees in California during 1950-2099. Plos One 4(7):1-9.

Luna, V., E. Lorenzo, H. Reinoso, M.C. Tordable, G. Abdala, R.P. Pharis, and R. Bottini. 1991. Dormancy in Peach (*Prunus persica* L.) Flower Buds. II. Comparative morphology and phenology in floral and vegetative buds, and the effect of chilling and gibberellin A3. Trees 5:244–246.

Magness, J.R. and H.P. Traub. 1941. Climatic adaptation of fruit and nut crops, p. 400-420. Yearbook of Agriculture. Univ. Press of the Pacific, Honolulu, Hawaii.

Marchi, S., L. Sebastiani, R. Gucci, and R. Tognetti. 2005. Sink-source transition in peach leaves during shoot development. J. Amer. Soc. Hort. Sci. 130(6)928-935.

Martínez-Gómez, P., F. Dicenta, D. Ruiz, and J. Egea. 2002. Flower bud abscission in apricot: Competition between vegetative and flower buds, and effects of early defoliation and high pre-blossom temperatures. J. Hort. Sci. Biotech. 77:485-488.

Martínez-Gómez, P., R. Sánchez-Pérez, and F. Dicenta. 2006. Fruit development in almond. Acta Hort. 726:241-246.

Martínez-Gómez, P., R. Sánchez-Pérez, F. Dicenta, W. Howad, P. Arús, and T.M. Gradziel. 2007. Almond, p. 229-242. In: C. Kole (ed.). Fruits and nuts. Genome mapping and molecular breeding in plants, vol. 4. Springer, Berlin, Germany.

Martínez-Gómez, P., R. Sánchez-Pérez and F. Dicenta. 2008. Fruit development in almond for fresh consumption. J. Am. Pomol. Soc. 62(2):82-86.

Micke, W.C. and D.E. Kester. 1997. Almond growing in California. Acta Hort. 470:21-28.

Mitchell, P. 1961. Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. Nature 191:144-148.

Møller, I.A., J. Browse, A. Madlung, and A.G. Rasmusson. 2018. Respiration and lipid metabolism, p. 317-353. In: L. Taiz, E. Zeiger, I.A. Møller, and A. Murphy (eds.). Plant physiology and development. International sixth addition. Oxford Univ. Press, NY, USA.

Morimoto, F.R. and K, Kumashiro. 1978. Studies on the rest-breaking of buds of deciduous fruit trees by chemical treatment. J. Fac. Agric., Shinshu Univ. 15:1-18.

Navorsingsinstituut vir Vrugte en Vrugtegnologie. 1985. Inligtingsbulletin nommer 545: Amandelverbouing. NIVV, Stellenbosch, South Africa.

Nicholls, D.G. 1982. Bioenergetics: An introduction to the chemiosmotic theory. Academic Press, NY, USA.

Nir G., J. Shulman, L. Fanberstein and G. Lavee. 1986. Changes in the activity of catalase in relation to the dormancy of grapevine. Plant Physiol. 81(4):1140-1142.

North, M.S. 1992. Alternative rest-breaking agents to DNOC/oil for apples. S. Afr. J. Plant Soil 9:39-40.

North, M.S. 1995. New rest-breaking agents for the control of delayed foliation of apples. Acta Hort. 409:151-153.

North, M.S. 2003. Alternative rest-breaking agents evaluated on 'Golden Delicious' apple trees. S. Afr. J. Plant Soil 20:59-63.

North, M.S., K. de Kock, N. Cook, L. Allderman, and M. Booyse. 2012. Effect of application date of rest breaking agents on growth and production of ‘Royal Gala’ and ‘Granny Smith’ apple. Acta Hort. 940:483-490.

Okkie, W.R. and D.J. Werner. 1996. Genetic influence on flower bud density in peach and nectarine exceeds that of environment. HortScience 31:1010–1012.

Olmsted, C.E. 1951. Experiments on photoperiodism, dormancy, leaf age and abscission in sugar maple. Bot. Gaz. 112:305-393.

Ortega, E., J. Egea, and F. Dicenta. 2004. Effective pollination period in almond cultivars. HortScience 39:19–22.

Patterson, B.D., L.A. Payne, Y.Z. Chen, and D. Graham. 1984. An inhibitor of catalase induced by cold in chilling-sensitive plants. Plant physiol. 76:1014-1018.

Pettis, J.S. and K.S. Delaplane. 2010. Coordinated responses to honey bee decline in the USA. Apidologie 14:256-263.

Pimienta, E. and V.S. Polito. 1982. Ovule abortion in 'Nonpareil' almond (*Prunus dulcis* (Mill.) D.A. Webb). Am. J. Bot. 69:913-920.

Pimienta, E. and V.S. Polito. 1983. Embryo sac development in almond (*Prunus dulcis* (Mill.) D.A. Webb) as affected by cross-, self and non-pollination. Ann. Bot. 51:469-479.

Pimienta, E., V.S. Polito, and D.E. Kester. 1983. Pollen tube growth in cross- and self-pollinated 'Nonpareil' almond. J. Amer. Soc. Hort. Sci. 108:643-647.

Polito, V., W.C. Micke, and D.E. Kester. 1996. Bud development, pollination and fertilization, p. 98-102. In: W.C. Wicke (ed.). Almond production manual. UCANR Publications, CA, USA.

Potts, S.G., J.C. Biesmeijer, C. Kremen, P. Neumann, O. Schweiger, and W.E. Kunin. 2010. Global pollinator declines: trends, impacts and drivers. Trends Ecol. Evol. 25:345-353.

Prudencio, A.S., P. Martínez-Gómez, and F. Dicenta. 2018. Evaluation of breaking dormancy, flowering and productivity of extra-late and ultra-late flowering almond cultivars during cold and warm seasons in South-East of Spain. Sci. Hortic. 235:39-46.

Quinn, N.R. 1941. Almond culture in South Australia. Dept. of Agric., Adelaide, Australia.

Rattigan, K. and S.J. Hill. 1986. Relationship between temperature and flowering in almond. Aust. J. Exper. Agric. 26:399–404.

Rattigan, K. and S.J. Hill. 1987. Relationship between temperature and flowering in almond: Effect of location. Aust. J. Exp. Agric. 26(3):905–908.

Reighard, G. L. and F. Loreti. 2008. Rootstock development, p. 193-220. In: D.R. Layne and D. Bassi (eds.). The peach: botany, production and uses. CABI, Boston, MA.

Reinoso, H., V. Luna, R.P. Pharis, and R. Bottini. 2002. Dormancy in peach (*Prunus persica*) flower buds. V. Anatomy of bud development in relation to phenological stage. Can. J. Bot. 80:656–663.

Richardson, E.A., S.D. Seely, and D.R. Walker. 1974. A model for estimating the completion of rest for 'Redhaven' and 'Elberta' peach trees. HortScience 9:331-332.

Richardson, E.A., S.D. Seeley, D.R. Walker, J.L. Anderson, and G.L. Ashcroft. 1975. Phenoclimatography of spring peach bud development. HortScience 10, 236–237.

Rodrigo, J. and M. Herrero, 2002. Effects of pre-blossom temperatures on flower development and fruit set in apricot. Sci. Hortic. 29:125-135.

Sáez, A., M.A. Aizen, S. Medici, M. Viel, E. Villalobos and P. Negri. 2020. Bees increase crop yield in an alleged pollinator-independent almond variety. Nature Research 10:1-7.

Sagredo, K.X., K.I. Theron and N.C. Cook. 2005. Effect of mineral oil and hydrogen cyanamide concentration on dormancy breaking in 'Golden Delicious' apple trees. S. Afr. J. Plant Soil 22(4):251-256.

Samish, R.M. 1945. The use of dinitrocresol minreal oil sprays for the control of prolonged rest in apple orchards. J. Pomol. Hort. Sci. 21:164-179.

Samish, R.M. 1954. Dormancy in woody plants. Ann. Rev. Plant Physiol. 5:183-203.

Samish, R.M. and S. Lavee. 1962. The chilling requirement of fruit trees. Publication of the Natl. Univ. Inst. Agr. 511:372-388.

Saure, M.C. 1985. Dormancy release in deciduous fruit trees. Hort. Rev. 7:239-300.

Segura, J.M.A., R. Socias i Company, and O. Kodad. 2017. Late-blooming in almond: A controversial objective. Scientia Hort. 224:61–67.

Shaltout, A.D. and C.R. Unrath. 1983. Rest completion prediction model for 'Starkrimson Delicious' apples. J. Amer. Soc. Hort. Sci. 108:957-961.

Sánchez- Pérez, R., F. Dicenta, and P. Martínez-Gómez. 2012. Inheritance of chilling and heat requirements for flowering in almond and QTL analysis. Tree Genetics & Genomes 8:379-389.

Sheard, A.G. 2008. Factors leading to poor fruit set and yield of sweet cherries in South Africa. Ph.D. dissertation, Univ. of Stellenbosch, Stellenbosch, South Africa. Date last accessed: 10 December 2020. http://library.sun.ac.za/en-za/Pages/Home.aspx

Sheard, A.G., S.D. Johnson, and N.C. Cook. 2009. Effect of timing and concentration of rest breaking agents on budburst in ‘Bing’ sweet cherry under conditions of inadequate winter chilling in South Africa. S. Afr. J. Plant Soil. 26(2):73-79.

Shiva S. 2013. Nitrite: A physiological store of nitric oxide and modulator of mitochondrial function. Redox Biol.1:40-44.

Shulman, Y., G. Nir, and S. Lavee. 1986. Oxidative processes in bud dormancy and the use of hydrogen cyanamide in breaking dormancy. Acta Hort. 179:141-148.

Siller-Cepeda, J.H., L.H. Fuchigami, and T.H.H. Chen. 1992. Hydrogen cyanamide-induced bud break and phytotoxicity in 'Redhaven' peach buds. HortScience 27:874-876.

Snir, I. 1983. Chemical dormancy breaking of red raspberry. HortScience 18:710-713.

Snir, I. and A. Erez. 1988. Bloom advancement in sweet cherry by hydrogen cyanamide. Fruit Var. J. 42:120-121.

Socias i Company, R. 1983. Flower sterility in almond. Acta Hort. 139:69-74.

Socias i Company, R. 1990. Breeding self-compatible almonds. Plant Breeding Rev. 8:313-338.

Socias i Company, R. 2017. Pollen-style (in)compatibility: development of autogamous cultivars, p. 188-208. In: R. Socias i Company and T.M. Gradziel (eds.). Almond: botany, production and uses. CABI, Boston, MA.

Socias i Company, R. and A.J. Felipe. 1992a. Almond: a diverse germplasm. HortScience 27:717–718.

Socias i Company, R. and A.J. Felipe. 1992b. Self-compatibility and autogamy in 'Guara' almonds. J. Hort. Sci. 67:313-317.

Socias i Company, R. and A.J. Felipe. 1994. Flower quality and fruit quality in almond: Conflicting objectives? p. 245-248. In: H, Schmidt and M. Kellerhals (eds.). Progress in Temperate Fruit Breeding, vol. 1, Springer, Dordrecht, Netherlands.

Socias i Company, R., D.E. Kester, and M.V. Bradley. 1976. Effects of temperature and genotype on pollen tube growth of some self-compatible and self-incompatible almond cultivars. J. Amer. Soc. Hort. Sci. 101:490-493.

Socias i Company, R., A.J. Felipe, J. Gómez Aparisi, J.E. García, and F. Dicenta. 1998. The ideotype concept in almond. Acta Hort. 470:51–56.

Socias i Company, R., A.J. Felipe, and J. Gómez Aparisi. 1999. A major gene for flowering time in almond. Plant Breed. 118:443–448.

Socias, R., J.M. Alonso, and J.G. Aparisi. 2004. Fruit set and productivity in almond as related to self-compatibility, flower morphology and bud density. J. Hort. Sci. Biotechnol. 79:754-758.

Socias i Company, R., J.M. Ansón and M.T. Espiau. 2017. Taxonomy, botany and physiology, p. 1-42. In: R. Socias i Company and T.M. Gradziel (eds.). Almond: botany, production and uses. CABI, Boston, MA.

Sparks, D. 1993. Chilling and heating model for pecan bud break. J. Amer. Soc. Hort. Sci. 118:29–35.

Stassen, P.J.C. 2007. Rootstocks for peaches and nectarines. ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa. Date last accessed: 11 December 2020. https://www.hortgro-science.co.za/wp-content/uploads/docs/2018/08/Rootstocks-for-peaches-and-nectarines.pdf

Stephenson, A.G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. Ann. Rev. Ecol. Syst. 12:253-279.

Sumner, D.A., W.A. Matthews, J. Medellín-Azuara, and A. Bradley. 2014. The economic impacts of the California almond industry. UC Agric. Issues Center, CA, USA. Date last accessed: 2 December 2020

http://aic.ucdavis.edu/almonds/Economic%20Impacts%20of%20California%20Almond%20Industry_Full%20Report_FinalPDF_v2.pdf

Swartz, H.J. and L.E. Powell. 1981. The effect of long chilling requirement on time of bud break in apple. Acta Hort. 120:173-178.

Szabó, Z., J. Nyéki, I. Szél, A. Pedryc, and A. Szalay. 1998. Low temperature injury in peach and nectarine cultivars. Acta Hort. 465:339–404.

Szalay, L. 2006. Comparison of flower bud development in almond, apricot and peach genotypes. Int. J. Hort. Sci. 12(2):93-98.

Taiz, L. 2018. Plant senescence and cell death, p. 665-692. In: L. Taiz, E. Zeiger, I.A. Møller, and A. Murphy (eds.). Plant physiology and development. International sixth addition. Oxford Univ. Press, NY, USA.

Taiz, L., G.B. Seymour, and H. Sze. 2018. Gametophytes, pollination, seeds, and fruits, p. 513-552. In: L. Taiz, E. Zeiger, I.A. Møller, and A. Murphy (eds.). Plant physiology and development. International sixth addition. Oxford Univ. Press, NY, USA.

Tao, R. and A.F. Iezzoni. 2010. The S-RNase-based gametophytic self-incompatibility system in Prunus exhibit distinct genetic and molecular features. Scientia Hort. 124:423-433.

Terblanche, J.H. and D.K. Strydom. 1973. Effects of autumnal nitrogen nutrition, urea sprays and a winter restbreaking spray on bud break and blossoming of young 'Golden Delicious' trees grown in sand culture. Decid. Fruit Grow. 23:8-14.

Theron, K.I., H.J. Gerber, and W.J. Steyn. 2011. Effect of hydrogen cyanamide, mineral oil and thidiazuron in combination with tip pruning on bud break, shoot growth and yield in ‘Bourjasotte Noire’, ‘Col de Damme Noire’ and ‘Noire de Caromb’ figs. Scientia Hort. 128:239-248.

Thomas, D. and J. Connell. 2018. Assessing phenology of almonds. PIRSA, Adelaide, Australia. Date last Accessed: 29 April 2020. https://2q1ee4456oc52trll42uctl1-wpengine.netdna-ssl.com/wp-content/uploads/2018/08/Assessing-Phenology-in-Almonds-27June-2018.pdf

Thorp, R.W. 1996. Bee Management for Pollination, p. 132-154. In: W.C. Wicke (ed.). Almond production manual. UCANR Publications, CA, USA.

Tombesi, S., B.D. Lampinen, S. Metcalf, and T.M. DeJong. 2016. Yield in almond is related more to the abundance of flowers than the relative number of flowers that set fruit. Calif. Agric. 71(2):68-74.

United States Department of Agriculture. 2018. Fruit and tree nut data for 2017. USDA, Washington, D.C., USA. Date last accessed: 10 December 2020. https://www.ers.usda.gov/data-products/fruit-and-tree-nuts-data/fruit-and-tree-nuts-yearbook-tables/

Vargas, F.J., J. Clavé, M.A. Romero, I. Batlle, and M. Roviro. 1997. Autogamy studies on almond progenies. Acta Hort. 470:74-81.

Verdú, A., S. Izquierdo, and R. Socias i Company. 2017. Processing and industrialization, p. 460-482. In: R. Socias i Company and T.M. Gradziel (eds.). Almond: botany, production and uses. CABI, Boston, MA.

Vergara, R. and F.J. Pérez. 2010. Similarities between natural and chemically induced bud-endodormancy release in grapevine *Vitis vinifera* L. Scientia Hort. 125(4):648-653.

Vergara, R., F. Parada, S. Rubio and F.J. Perez. 2012. Hypoxia induces H_2O_2 production and activates antioxidant defence systems in grapevine buds through mediation of H_2O_2 and ethylene. J. Exp. Bot. 63(11):4123-4131.

Wang, J.Y. 1960. A critique of the heat unit approach to plant response studies. Ecology 41:785–790.

Wang, S.Y. and M. Faust. 1993. Comparison of seasonal growth and polyamine content in shoots of orchard-grown standard and genetic dwarf apple trees.

Wang, S.Y., G.L. Steffens, and M. Faust. 1986. Breaking bud dormancy in apple with a plant bioregulator, thidiazuron. Phytochemistry. 25:311-317.

Wareing, P.F. 1953. Growth studies in woody species. Photoperiodism in dormant buds of *Fagus sylvatica* L. Ibid. 6:692-706.

Watanabe, M.E. 1994. Pollination worries rise as honey bees decline. Science 265:1170-1171.

Weinbaum, S.A. 1985. Role of natural self-pollination in self-fruitfulness of almond. Scientia Hort. 27:295-302.

Weinbaum, S.A., D.V. Shaw, and T.T. Muraoka. 1989. Independence of self-compatibility and potentiality for self-pollination in peach × almond hybrids. Euphytica 41:53-58.

Werner, D.J., B.D. Mowrey, and J.X. Chaparro. 1988. Variability in flower bud number among peach and nectarine clones. HortScience 23:578–580.

Williams, R.R. 1965. The effect of summer nitrogen application on the quality of apple blossom. J. Hort. Sci. 40:31-41.

Winfree, R., R. Aguilar, D.P. Vázquez, G. LeBuhn and M.A. Aizen. 2009. A meta-analysis of bees' response to anthropogenic disturbance. Ecology 90:2068-2076.

Wirthensohn, M. and L. Iannamico. 2017. Almond in the southern hemisphere, p. 87-110. In: R. Socias i Company and T.M. Gradziel (eds.). Almond: botany, production and uses. CABI, Boston, MA.

Yamane, H. and R. Tao. 2009. Molecular basis of self-(in)compatibility and current status of *S*-genotyping in Rosaceous fruit trees. J. Japan. Soc. Hort. Sci. 78:137-157.

PAPER 1: Dormancy progression of 'Independence' almond trees under South African conditions

Additional index words. Bud break, low-chill, chill requirement, heat requirement, *Prunus dulcis*

Abstract.

Dormancy is a developmental phase in deciduous fruit trees that ensures their survival during unfavourable winter conditions. Dormancy is divided into three classifications, namely *endodormancy*, which is controlled solely by signal recognition within an affected structure; *paradormancy*, which is controlled by signalling from a structure other than the affected structure; and *ecodormancy*, where environmental conditions control the state of dormancy. The relationship between low temperatures and dormancy progression has led to the concept of a chill requirement necessary to overcome endodormancy. Various models, such as the Utah, Daily Positive Utah Chill Unit and Dynamic models, have been developed to estimate chill accumulation, assisting growers when matching cultivars to suitable environments. Almonds are well suited for production in Mediterranean-type climates, such as the Western Cape. However, low prices and poor yields, together with expensive equipment and machinery needed for harvesting and processing, have restricted local almond production in past, thereby preventing the establishment of a South African almond industry. Independence is a newly developed, self-compatible almond cultivar with a lower chill requirement compared to cultivars such as Nonpareil, with later flowering in September and early harvesting in February and March. The purpose of this study was to determine the dormancy progression and bud break patterns, as well as the chill and heat requirements needed to overcome dormancy, in 'Independence' almond trees grown in the Western Cape. Results suggest that dormancy in 'Independence' almond trees progressed independently from chill accumulation but showing a greater reliance on heat accumulation for subsequent flowering. Furthermore, the extremely low levels of dormancy in these almond trees resemble that of previous work done on low-chill plum, peach, and apricot cultivars in South Africa. The time of entrance into dormancy varied

greatly among eight different orchards and the two consecutive seasons, respectively, as did the chill requirements, while the exit from dormancy was more comparable among farms. This questions the suitability of the available chill models to accurately describe the progression of endodormancy in low-chill cultivars grown under mild winter conditions. An inverse relation between the chilling and heat accumulation required for flowering in 'Independence' almond trees occurred. From these results climatic conditions in the Western Cape are suitable for the commercial production of 'Independence' almond trees, with regards to chill and heat accumulation to ensure successful bud break in the subsequent spring.

Deciduous fruit trees enter a phase of dormancy during winter, inhibiting meristem activity and allowing the trees to withstand unfavourable climatic conditions such as short photoperiods and low temperatures (Faust et al., 1997). Together with assisting trees in enduring winter conditions, dormancy also ensures survival and reproduction by delaying flowering time and fruit set of individual trees (Campoy et al., 2011a). These authors stated that not all physiological processes cease during this developmental phase, and future growth and development is affected by physiological activities during dormancy. Lang et al. (1987) divided dormancy into three classifications, namely endodormancy, paradormancy and ecodormancy. Ecodormancy is described as the inhibition of visible growth imposed by unfavourable environmental factors (i.e. low temperatures); previously called quiescence. During paradormancy, growth initiation is controlled by a structure(s) other than the affected structure (bud), such as apical dominance; previously called correlative inhibition. Lastly, endodormancy refers to a dormant state controlled by signal recognition within the affected structure alone; the true dormancy (Lang et al., 1987). Endodormancy is a very complex phenomenon in deciduous fruit trees grown in temperate regions, and despite various investigations many questions remain (Campoy et al., 2011a).

Coville (1920) was the first to indicate the relationship between low temperatures and dormancy release. This led to the concept of a chilling requirement (CR) needed to overcome dormancy in deciduous fruit trees (Campoy et al., 2011a). This parameter is useful when determining the successful adaptation of a cultivar to any environment (Samish and Lavee, 1962); a factor that is of great economical importance when establishing a new orchard. However, CRs vary among cultivars, and chill accumulation between locations and seasons,

questioning the accuracy in determining the quantity of cold necessary to overcome dormancy (Campoy et al., 2011a). To address this problem, Richardson et al. (1974) developed the Utah model, assigning chill unit (CU) values to different temperature ranges. Adjustments made to the Utah model led to the development of other models such as the Low Chilling model (Gilreath and Buchanan, 1981) and the North Carolina model (Shaltout and Unrath, 1983).

An important milestone in dormancy modelling, was the development of the Dynamic model (Fishman et al., 1987a, 1987b) which addressed some inaccuracies of the Utah model when used in warm-winter regions (Campoy et al., 2011a). The Dynamic model expresses a fixed amount of chill accumulation as Chill Portions (CP) and incorporates how subsequent moderate temperatures (13°C to 15°C) can enhance the positive effect of a cold period (Erez and Couvillon, 1987; Guerriero et al., 1985). However, in a study by Balandier et al. (1993), none of the aforementioned models could accurately describe the phenology of peach under tropical conditions. The Daily Positive Utah Chill model, locally known as the "Infruitec" model, is an adaptation of the Utah model that is also aimed at improving the accuracy of chill accumulation under warmer winter conditions by excluding the chill negation mechanism within a 24-hour cycle (Linsley-Noakes et al., 1994). A study by Luedeling et al. (2009) on walnuts in California found the Dynamic and "Infruitec" models to be more successful in explaining phenology, compared to the Utah and Chilling Hours (hours between 0 – 7.2°C) model, but not necessarily more accurate when predicting phenological dates. Therefore, when determining CRs, factors such as tree behaviour, location and experimental conditions under which requirements were estimated, should also be considered (Campoy et al., 2010; Luedeling and Brown, 2011). Likewise, Campoy et al. (2011a) found that these models are used to represent the progression of dormancy, but lack a functional understanding of tree physiology.

Campoy et al. (2011b) found that the accumulation of both chill and heat is necessary for bud development, but expressed an uncertainty about the role of heat during dormancy. Richardson et al. (1975) argued that bud growth and development results from temperatures above a certain base level, after endodormancy completion. These authors assume that no growth will take place when ambient temperatures are below the base level, while a linear increase in growth will be observed as temperatures rise above this level. The Growing Degree Hour (GDH) model was therefore developed with a base temperature of 4.5°C and 25°C as the upper limit, which allows for an approximation of when certain bud growth and development stages will take place after endodormancy completion in peaches (Richardson et al., 1975). Therefore, the so-called heat requirement (HR) can be determined by observing the response

of a tree to heat accumulation, above a threshold temperature, from the end of dormancy until flowering (Anderson et al., 1986; Richardson et al., 1974).

The CR and HR work synergistically to prevent trees from sprouting during unfavourable conditions in winter, while ensuring bud break early enough for trees to complete their annual cycle (Luedeling, 2012). South African deciduous fruit growing regions have mild winter conditions, generally characterized by high average day temperature (Strydom et al., 1971; Linsley-Noakes et al., 1995). This often leads to insufficient winter chill causing incomplete endodormancy release resulting in delayed and low bud break that is protracted and unsynchronised (Erez, 2000). One adaptation strategy to address the challenges associated with insufficient chill, is breeding cultivars with low CRs (Luedeling, 2012). Independence is a self-compatible almond cultivar developed by Zaiger's Inc. Genetic from a cross between the All-In-One cultivar and Almond selection 2168 (Batlle et al., 2017). Characteristics such as a lower CR of approximately 23.7 CP according to the Dynamic model (Cape Almonds, 2017), abundant flowers during bloom and excellent yield (Batlle et al., 2017) has made Independence a popular cultivar in the USA. Since its release in 2008, the cultivated hectares of 'Independence' in the USA have increased from 16 ha to nearly 2000 ha in a decade (California Department of Food and Agriculture, 2019). Likewise, Independence as a cultivar shows a growing popularity under South African growers. Cape Almonds (ZZ2) received the exclusive master-licence to cultivate 'Independence' in the Southern Hemisphere from Zaiger's Inc. Genetics and Zaiger SA in 2016. Since then, Cape Almonds, in partnership with various sub-licensee growers, have planted more than 600 ha of 'Independence' almond trees throughout the Western Cape Province (personal communication, Cape Almonds).

Characteristics such as a relatively low CR, tolerance to heat and drought during summer and early, rapid shoot growth makes almonds well suited for a Mediterranean-type region with mild winter conditions and dry, hot summers (Gradziel et al., 2017) such as parts of the Western Cape. Inquiries into the suitability of the Western Cape as a commercial almond production region dates as far back as 1980, when Dr G Kochba, an almond expert from Israel, was invited to investigate the feasibility of almond cultivation under South African conditions (Navorsingsinstituut vir Vrugte en Vrugtegnologie, 1985). Therefore, the objective of this study is to investigate the dormancy progression and bud break patterns, as well as the CR and HR in various commercial 'Independence' almond orchards in the Western Cape, South Africa.

Materials and Methods

Plant material and site description. Forcing trials were conducted over two consecutive seasons on shoots from three- and four-year-old 'Independence' almond trees on 'Viking' rootstocks, sourced from eight commercial orchards (Table 1) representing the current almond growing regions of South Africa. During the 2020 season, only four of the eight commercial orchards could be included in the trial (Table 1).

Each week, thirty, one-year-old shoots (±40 cm) were randomly selected in each orchard throughout the dormant period. Shoot collection commenced at bud set and was terminated when natural bud swell occurred. The sampling was performed from week 8 to week 33 for the first season and week 1 to week 32 during the second season. To prevent dehydration, leaves were manually removed from shoots in the orchard by cutting through the middle of the petiole in order to prevent damage to the shoot and buds. Shoots were then placed in plastic bags (standard commercial courier bags) and transported to the laboratory within 72 hours. All sampling dates were recorded together with the latitude and altitude of each orchard. Temperature data were obtained for each orchard by placing Tinytag Plus 2 data loggers (Gemini Data Loggers, UK) in each study site recording the hourly temperature for the duration of the trial.

Trial lay-out, forcing conditions and data collection. Upon arrival at the laboratory, the shoots were cut back to 30 cm by removing excess basal shoot tissue. The shoots were randomly divided into three bundles of ten shoots each (thus, three replicates per orchard), labelled, dipped in 5 cm^3 of household bleach (3.5% sodium hypochlorite) and placed in white plastic buckets containing 1 dm^3 of water. The buckets containing the shoots were placed in three identical Snijders Scientific growth chambers (Economic Delux, ECD01E, Tilburg, Netherlands) that maintained a constant 25 °C (± 1˚C) and continuous illumination (ca. 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation). The water in the buckets was replaced every second day and 10 mm of the basal part of each shoot was removed weekly to prevent xylem blockage. The shoots were monitored every second day. The number of days to 50% bud break was recorded when five of the shoots in a bundle had a single bud (either terminal or lateral) showing signs of bud break. The time interval between the commencement of the forcing and 50% bud break was used as an indication of the depth of dormancy. Shoots were kept in the growth chambers for 80 days or until 50% bud break occurred. The average daily temperature obtained from each logger was used to calculate the accumulated CUs/CPs according to the

Utah, Infruitec and Dynamic models, as well as the accumulated heat units according to the Growing Degree Hours (GDH) model with a base temperature of 4.5°C and 10°C.

Dormancy progression. The dormancy progression of each orchard was determined by plotting individual scatter graphs representing the dormancy level (days to 50% bud break) for each of the collection dates (see Appendix A). A typical dormancy progression curve should contain a period where the dormancy levels increase (entrance into dormancy), reach a maximum and then decrease (exit from dormancy). The scatterplots were modelled by fitting two linear joint line models which represented the entrance into dormancy and exit from dormancy with the joint point signifying the maximum depth of endodormancy (as seen in Fig. 1). Trees are considered "dormant" when more than ten days under forcing conditions are required for shoots to reach 50% bud break; likewise, when shoots reached 50% bud break within ten days of forcing, trees have overcome dormancy. The modelling involved a univariate, nonlinear regression analysis performed with SAS statistical software similar to that used in Cook et al. (2017).

The model can be described as two converging straight lines:

$$\text{Dormancy Entrance} = a_1 + b_1\,(\text{Day of Year})$$

$$\text{Dormancy Exit} = a_2 + b_2\,(\text{Day of Year})$$

Where $a_2 = a_1 + (b_1\text{-}b_2)$ (Day of Year) and Day of Year = Joining point

The following five parameters were determined for each orchard from the dormancy progression models fitted for each season: the day of year (DOY) when dormancy is entered; the induction period (number of days between entering dormancy and reaching maximum depth of dormancy); when maximum dormancy levels was reached, the depth of dormancy (number of days to 50% bud break when at maximum level of dormancy), as well as the DOY of dormancy release for each orchard, respectively (see Fig 1).

Evaluation of chill and heat requirements. Chill and heat requirements were calculated similar to the methods of Prudencio et al. (2018). The chill accumulation for each farm was determined by the endodormancy period. The endodormancy period is the number of days between the start of chill accumulation, selected as the date at which the Utah model no longer negated CU to zero, and the date of dormancy breaking (50% of shoots in each bundle showing signs of bud break within ten days of forcing). The CR was then calculated by the number of CU accumulated during the endodormancy period. Likewise, the heat accumulation was

determined during the ecodormancy period. The ecodormancy period is the number of days between dormancy breaking (50% of shoots in each bundle showing signs of bud break within ten days of forcing) and the full bloom date (80% flowering) of each orchard in the field. The HR was then calculated as the number of heat units accumulated during the ecodormancy period.

The temperature data were used to represent the daily maximum and minimum temperatures (°C) (Appendix B) and to calculate the chill accumulation according to the Utah (Richardson et al., 1974), "Infruitec" (Linsley-Noakes et al., 1994) and Dynamic (Fishman et al., 1987a, 1987b) models, while heat accumulation was done according to the Growing Degree Hour (Richardson et al., 1975) model.

Statistical analysis. The data were analyzed with SAS Enterprise guide 7.1 (SAS Institute Inc., Cary, North Carolina, USA) using the linear model procedure and the pairwise t-test to determine the least significant difference (LSD) when the F-statistic indicated significance of $p<0.05$.

Note: During the 2019 season, the two-line modelling could not be performed for two of the farms (namely Kruispad and Hex Poort) due to insufficient data points at the start of the collection period, thus dormancy start date, induction period, DOY of maximum dormancy, as well as depth of dormancy could not be determined. In the 2020 season shoot collection commenced earlier ensuring the modelling and accurate calculations for these values. Due to the global Covid-19 pandemic, the courier services that collected and delivered the shoots from the various farms could not operate at full capacity, and thus only the nearest farms could have been serviced during the 2020 trial.

Results

Results from the 2019 season:

Dormancy progression. When considering the dormancy curves of the various orchards (Fig. 2), significant differences ($p<0.0001$) were found in the date of dormancy entrance (Table 2), ranging from the beginning of March to mid-May for the respective farms. Major's Drift was the first orchard to enter dormancy, although the entry date did not differ significantly from Amanteco and Groenrivier. Welverdiend was the fourth orchard to enter dormancy, the

entry date differing significantly from all orchards. Doornbos and Tamarak entered dormancy at a significantly later date, compared to all the farms. The induction period ranged between 47.5 – 58.3 days for all the farms except Tamarak, where the induction period was significantly shorter (p=0.0183) at 15.9 days (Fig. 2 and Table 2).

The day of the year when maximum dormancy (Fig. 2) was reached differed significantly (p=0.0038) among the different orchards (Table 2). Groenrivier, Major's Drift and Amanteco were the first farms to reach maximum dormancy, however, Amanteco did not differ significantly from Tamarak and Kruispad. The day of the year when maximum dormancy was reached for Doornbos did not differ significantly from that Tamarak and Welverdiend. Maximum dormancy levels were reached from mid-April to mid-June for the respective farms, generally prior to any chill accumulation, according to the date determined by the Utah model (arrows on Fig. 2).

Even though significant differences (p=0.0090) were found for the maximum depth of dormancy among the different orchards (Table 2), none of the orchards exceeded 25 days to 50% bud break, indicating a superficial dormancy level for 'Independence' almond trees. Groenrivier, Major's Drift and Amanteco had the highest number of days to 50% bud break at maximum dormancy level. The depth of dormancy in Groenrivier and Amanteco did not differ significantly from that of the Welverdiend farm, which, in turn, did not differ from Doornbos and Tamarak.

The day of the year the orchards exited from dormancy also showed significant differences (p=0.0067) amongst the different orchards (Fig. 2 and Table 2), ranging from mid-June to mid-July, respectively. Tamarak was the first farm to exit from dormancy but did not differ significantly from Amanteco. Amanteco, in turn, did not differ significantly from any of the other farms, with the exception of Kruispad, showing a significantly later date for exiting dormancy. Kruispad did not differ significantly from Groenrivier, Hex Poort, Doornbos and Welverdiend.

The endodormancy period varied between 1.4 – 62.3 days, s differing significantly (p<0.0001) among the different farms (Table 2). Kruispad had a significantly longer endodormancy period, compared to all the other farms. Major's Drift, Amanteco, Doornbos and Welverdiend did not differ significantly from each other, while Groenrivier, Hex Poort and Tamarak had a significantly shorter endodormancy period. According to the Utah model, Amanteco, Major's Drift and Kruispad started chill accumulation from 17 May – 22 May 2019,

which is noticeably earlier compared to the rest of the farms, starting from 12 June – 24 June 2019, respectively (Fig. 3 and arrows in Fig. 2).

No significant difference was found in the ecodormancy period among the different farms, ranging between 47.2 – 68.2 days (Table 2). The start of heat accumulation ranged from 16 June – 21 July 2019, corresponding with the date of dormancy exit and concluded when each farm reached their respective full bloom dates (Fig. 4), ranging from 22 Aug – 8 Sep 2020.

Chill and heat accumulation and requirements. During the 2019 season, the chill accumulation ranged between 17.2 – 182.2 CU, 17.2 – 268.0 CU and 0.4 – 16.7 CP for the Utah, "Infruitec" and Dynamic models, respectively and differed significantly among farms (Table 3). According to the Utah model, Amanteco had a significantly higher chill accumulation, compared to the rest of the farms. Doornbos, Kruispad, Groenrivier Major's Drift, Hex Poort and Welverdiend, did not differ significantly from each other. Tamarak showed the lowest chill accumulation according to the Utah model, but did not differ significantly from that of Groenrivier, Major's Drift, Hex Poort and Welverdiend. According to the "Infruitec" model, Amanteco and Kruispad accumulated significantly more CUs, compared to the rest of the farms, followed by Major's Drift, Doornbos and Welverdiend, that did not differ significantly from each other. Doornbos did not differ significantly from Groenrivier, while Welverdiend did not differ significantly from Groenrivier or Hex Poort, which in turn did not differ significantly from Tamarak, again showing the lowest chill accumulation. For the Dynamic model, Kruispad and Amanteco showed the highest chill CP accumulation, while the CP accumulation for Major's Drift did not differ significantly from that of Amanteco. The CP accumulation for Groenrivier, Doornbos and Welverdiend did not differ significantly from each other or from that of Hex Poort that in turn, did not differ from Tamarak, again showing the lowest CP accumulation.

The heat unit (HU) accumulation for the different farms ranged between 11158 – 13999 GDH and 4360 – 5832 GDH for models with a base temperature of 4.5°C and 10°C, respectively. However, no significant differences were found for the HU accumulation among the various farms for both models (Table 3).

Results from the 2020 season

Dormancy progression. No significant differences were found in the day of the year of entrance into dormancy among the different farms (Table 4). The starting date ranged from the beginning of February to the end of March 2020 for the respective farms (Fig. 5).

No significant differences were found in the induction period among various farms (Table 4). The length of the induction period ranged between 67.6 – 101.0 days for the respective farms (Fig. 5). A significant difference (p=0.0331) was, however, found in the DOY of maximum dormancy among different farms (Table 4). Major's Drift and Hex Poort were the only two farms differing significantly from each other (Fig. 5). No significant differences were found for the maximum level of dormancy among the different farms (Table 4). However, it should be noted that none of the farms exceeded 30 days to reach 50% bud break when at maximum depth of dormancy (Fig. 5).

The day of the year of dormancy breaking differed significantly (p=0.0207) amongst the orchards (Table 4), ranging from the end of June to mid-July 2020, respectively. Amanteco was the first orchard to exit dormancy but did not differ significantly from Major's Drift. Groenrivier was the last to exit dormancy but did not differ significantly from Hex Poort, while Major's Drift and Hex Poort did not differ significantly from each other (Fig. 5).

The duration of the endodormancy period was between 21.3 - 52.7 days, differing significantly (p=0.0019) for the various farms (Table 4). Hex Poort had a significantly shorter endodormancy period, compared to the other three farms. Major's Drift had a significantly longer endodormancy period compared to Groenrivier, while Amanteco did not differ significantly from either of the aforementioned. According to the chill models, chill accumulation for Major's Drift and Amanteco started 16 May 2020, a month earlier than at Groenrivier and Hex Poort, starting from 13 June – 29 June 2020, respectively (arrows on Fig. 5 and Table 5). No significant differences were found in the ecodormancy period among the four farms. Heat accumulation started from 2 July – 21 July 2020, with Amanteco and Major's Drift again starting earlier than Groenrivier and Hex Poort (Fig. 6).

Chill and heat accumulation. The chill accumulation ranged between 49.3 – 346.5 CU, 143.3 – 269.0 CU and 10.2 – 12.8 CP for the Utah, "Infruitec" and Dynamic models, respectively. Significant differences were found for the CU accumulation according to the Utah (p<0.0001) and "Infruitec" (p=0.0034) model, among the various farms (Table 5). According to the Utah model, Hex Poort had a significantly higher CU accumulation, compared to the rest of the farms, followed by Amanteco, showing a significantly higher CU accumulation than that

of Major's Drift while for Groenrivier it the lowest. According to the "Infruitec" model, Groenrivier again had a significantly lower CU accumulation, compared to the rest of the farms that did not differ significantly from each other. The CP accumulation according to the Dynamic model did not differ significantly among the various farms.

The HU accumulation for the different farms ranged between 9773 – 12561 GDH and 3830 – 5144 GDH for the respective models with a base temperature of 4.5°C and 10°C. However, no significant differences were found for either model in the GDH accumulation among the different commercial farms (Table 5).

Discussion

In *Prunus* species, reproductive bud break takes place prior to vegetative bud break under natural conditions (Alonso, 2017). Conversely, under forcing conditions in our study, vegetative buds started breaking before the reproductive buds. Therefore, our dormancy progression results were based on vegetative bud break as reproductive bud break did not take place until very close to endodormancy release. These results are congruent with findings by Luna et al. (1991) indicating that reproductive peach buds were unresponsive to forcing conditions, even after gibberellic acid (GA_3) or chill treatments. These authors went on to study the morphological development of the buds and concluded that final floral development remains incomplete up to a few days before natural bud break, compared to vegetative buds that were already fully developed by midsummer/early autumn. Thus, during forcing experiments, growth inhibition of the reproductive buds was not related to chill accumulation but rather to a morphological immaturity/incompleteness and chill requirement could only be calculated based on the response from the vegetative buds.

Dormancy induction varied greatly among the different commercial farms throughout the Western Cape, mainly due to the difference in date of endodormancy entrance for the respective farms, which could not be ascribed to any of environmental factors measured. The induction period, however, was more similar among the farms, except for Tamarak, having a significantly shorter induction period. The day of the year that maximum dormancy was reached, corresponded with the date of endodormancy entrance, with the exception of Tamarak, due to its significantly shorter induction period. Likewise, orchards with an earlier entrance date showed a higher maximum level of endodormancy. Contradicting findings were indicated for dormancy induction in apricot (Campoy et al., 2011c) and apple (Cook and Jacobs, 2000), with regards to ambient temperatures. Campoy et al. (2011c) indicated faster dormancy

induction in regions with higher minimum temperatures, while Cook and Jacobs (2000) found that apples grown in a colder region reached maximum dormancy levels earlier, compared to those grown in warmer conditions. However, neither of these studies correspond with our results for dormancy induction in 'Independence' almond trees grown throughout the Western Cape, as differences in endodormancy progression could not be ascribed to differences in chill accumulation. The four farms sampled in both seasons indicated seasonal differences with dormancy induction starting one week later in 2020 and an endodormancy period up to 28 days longer, compared to the previous season. Extended dormancy induction can be ascribed to differences in the daily maximum and minimum temperatures for the two seasons. Temperature data (Appendix B) indicated a more moderate average daily temperature in the 2019 season, compared to a greater difference in daily maximum and minimum temperatures experienced during the 2020 season. Moderate temperatures during the first season could have served as an environmental queue, inducing an earlier entrance into dormancy (Tanino et al., 2010), compared to the second season.

Our results generally indicated that orchards entering endodormancy early, reached their maximum dormancy level quicker, and showed a deeper dormancy level, compared to orchards entering later. However, it should be highlighted that none of the orchards exceeded 30 days to reach 50% bud break depicting an extremely low level of endodormancy for 'Independence' almond trees. This is in accordance with results from Cook (2010) showing less than 30 days to reach 50% bud break when at maximum level of dormancy for plum cultivars (*Prunus salicina* Lindl.*)* such as Ruby Red, Angelino, African Delight and Southern Bell, as well as peach cultivars such as Alpine and August Red. Likewise, Campoy et al. (2011c) indicated that none of the seven apricot *(Prunus armeniaca* L.*)* cultivars investigated, with CRs ranging from very low to low and medium, exceeded 30 days to reach 30% bud break, when grown under South African conditions. However, results for sweet cherries (*Prunus avium* L.) cultivated in South Africa ranged between 78 – 187 days to reach 50% bud break when at maximum dormancy level under inadequate winter conditions (Kapp, 2008).

In our study, orchards reached their maximum dormancy level generally prior to any chill accumulation according the three chill models, suggesting that environmental factors, other than chill, play a key role in dormancy induction in *Prunus.* Possible factors could be decreasing minimum temperature and shorter photoperiods (Campoy et al., 2011c; Heide, 2008) or simply genetically driven. Campoy et al. (2011c) stated that it would be incorrect to assume that dormancy induction in warmer growing regions results solely from chill

accumulation. Even though dormancy induction, as well as the maximum level of dormancy, varied substantially among the different commercial farms, it is evident from Fig. 2 and 3 that endodormancy breaking was less variable and more similar among orchards. Growth inhibition during dormancy induction is a combination of intra-plant relationships whereby paradormancy is gradually replaced by endodormancy (Faust et al 1997). This state seems to be biologically more complex compared to dormancy release where endodormancy is replaced by ecodormancy and the plant reacts to abiotic, external signals. Considering our results, it could be suggested that almond trees use the induction period to re-adjust biological differences built up during the growing season and once maximum dormancy is reached they are biologically more comparable and react more similar to environmental cues. Additionally, our findings also propose that that the chill models used are better suited at predicting dormancy release, compared to dormancy induction, possibly due to environmental factors other than chilling regulating the induction phase. This emphasizes the concern raised by Campoy et al. (2011b) that these linear models, with temperature effect used as the only variable, might not be well suited for the complexities involved in characterising progression during the endodormant period, especially under mild winter conditions.

Therefor it is not surprising that studies on the influence of chill accumulation on dormancy progression in deciduous trees are riddled with contradicting results. Some studies indicated that earlier maximum dormancy levels are achieved in colder regions (Cook and Jacobs, 2000), while others stated that higher minimum temperatures (warmer regions) accelerated dormancy progression (Campoy et al., 2011c). However, dormancy progression results from this study showed that bud dormancy in ‘Independence’ almond trees progressed independently from the chill accumulation for the various orchards throughout the Western Cape, characterised by mild winter conditions.

The CR and HR calculations used in our study is similar to what was used in Prudencio et al. (2018) to determine the CR and HR of three self-compatible almond cultivars grown in Murcia, south-eastern Spain. Independence is a later flowering almond cultivar (Cape Almonds, 2017) showing similar endodormancy breaking and flowering times as the very late flowering almond cultivar, Penta, compared to that of the very early cultivars, Desmayo Largueta and the ultra-late cultivar, Tardona, studied by Prudencio et al. (2018). Result from our study indicated a similar endodormancy period for ‘Independence’ and ‘Desmayo Largueta’, which was shorter compared to that of ‘Penta’ and ‘Tardona’. The overall CR for ‘Independence’ was much lower compared to the average requirement of 167 – 638 CU (Utah

model) and 21 – 56 CP (Dynamic model) indicated for 'Desmayo Largueta', 'Penta' and 'Tardona', respectively (Prudencio et al., 2018). Furthermore, according to our studies, the CR proved to be much lower than the approximate 400 CU (Utah model) and 23.7 CP (Dynamic model) that has been proposed for 'Independence' almond trees cultivated in California (Cape Almonds, 2017).

However, the CU/CP accumulation varied greatly among the different commercial farms, as well as between seasons for each farm. This is in accordance with results from Campoy et al. (2011b) indicating a difference in the CR calculated for the low-chill apricot cultivar, Palsteyn, in spite of similar dates for the release of endodormancy for this cultivar in the seasons tested. These authors noted that low-chill cultivars, in particular, are more likely to experience annual differences in CRs within a single cultivar. They suggested that year-to-year variability may be due to variation in climatic conditions inducing different levels of maximum dormancy during the respective seasons, inaccurate calculations of chill accumulation due to imperfections in the chill models or a combination of the aforementioned factors. Furthermore, Campoy et al. (2011c), indicated a CR (using the Utah model) for 'Canino' apricots ranging from 304 CU under South African conditions to 806 CU when cultivated in Spain. The inconsistent CR shown in both these trial sites again raises the concern regarding the ability of the available chill models to accurately describe dormancy progression.

Our results indicated that the ecodormancy period for Independence was longer than any of the three almond cultivars studied by Prudencio et al. (2018). The HR for 'Independence' almond trees grown throughout the Western Cape ranged between 9773 -13999 GDH and 3830 – 5832 GDH for the respective models with a base temperature of 4.5°C and 10°C. These results are also higher than the 6279 – 8571 GDH (base temperature of 4.5°C) proposed for 'Desmayo Largueta', 'Penta' and 'Tardona', respectively (Prudencio et al., 2018).

A substantially lower CR, together with an evidently higher HR, for 'Independence' compared to 'Desmayo Largueta', 'Penta' and 'Tardona' (Prudencio et al., 2018), is evident of the inverse relationship between CR and HR for dormancy progression and flowering, as proposed by Sparks (1993) for pecans. Results from our study suggests that 'Independence' almond trees seem to be more reliant on sufficient heat accumulation during the ecodormancy period than chill accumulation during the endodormancy period, for successful bud break. That being said, HR for this study was determined once endodormancy breaking was reached for the respective farms. Campoy et al. (2011b) suggested that the accumulation of chill and heat

units occur simultaneously in the low-chill apricot cultivar, Palsteyn, once partial chill accumulation has been acquired. Therefore, heat accumulation most likely started prior to endodormancy release, possibly indicating an even greater reliance on HR for successful bud break in 'Independence' almond trees.

Dormancy progression results indicated that low-chill almond cultivar, Independence, is well suited for commercial production in the Western Cape, South Africa, with regards to climatic conditions. Andreini et al. (2012) ascribed the adaptability of low-chill apricot cultivars to unfavourable climatic conditions, to their ability to synchronise biological processes such as the release of dormancy, andregenesis and microsporogenesis, rather than purely satisfying the CR. These authors argue that cultivars with high CRs lack this synchronisation ability, leading to irregular growth of reproductive buds and subsequent failure of flowering and fruit set under inadequate winter chill. For future dormancy progression studies, the inclusion of this synchronisation phenomenon during chill accumulation, might lead to a better understanding of endodormancy progression under inadequate winter chill.

Conclusion

Even though our trials depicted dormancy progression in 'Independence' almond trees based only on vegetative bud break, the CRs calculated are in fact a conservative estimate of the whole tree, as some have speculated that reproductive buds typically have a lower CR compared to vegetative buds (Saure, 1985). However, it seemed as though the dormancy progression of 'Independence' almond trees proceeded, irrespective of chill accumulation.

The extremely low levels of dormancy depicted in Independence is not unusual for low-chill stone fruit cultivars under South African conditions, as found by Cook (2010) and Campoy et al. (2011b). However, the high variability in the entrance into dormancy, as well as variably chill accumulation, among orchards and between seasons highlight the complexity of (especially) dormancy induction and at the same time questions the ability of the available chilling models, to accurately describe endodormancy progression under mild winter conditions (Campoy et al., 2011c). Furthermore, flaws in the methodology of forcing experiments, such as a lack in uniformity of shoot types that were sampled, could contribute to the high variability indicated in the results. Results from this study are in accordance with research by Sparks (1993), indicating an inverse relationship between chilling and the heat

accumulation needed to overcome dormancy. Our results, therefore suggest that 'Independence' is more reliant on heat accumulation and less reliant on chilling to ensure successful endodormancy release and subsequent bud break.

The results from this study highlights the statement made by Campoy et al. (2011a) that "[chill] models are proxies for explaining dormancy overcoming, but without deep biological significance based on a functional understanding of tree physiology". We suggest an investigation into the synchronisation of biological processes as described in Andreini et al. (2012), together with chill accumulation, to better understand the course of endodormancy in almonds under South African conditions.

Marginal winter conditions do not seem to be a limiting factor to the commercial production of 'Independence' almond in the Western Cape, South Africa. With regards to climatic conditions, our study supports the conclusion of Dr G. Kochba (Navorsingsinstituut vir Vrugte en Vrugtegnologie, 1985) that the largest part of the Western Cape, with particular reference to the Piketberg district, Tulbagh and Calitzdorp regions, is suitable for commercial almond production, especially due to the absence of late winter frost. 'Independence' almond cultivation could present a plausible alternative to agriculture in areas of the Western Cape region faced with a rapidly changing climate due to increasing global temperatures.

Literature Cited

Alonso, J.M. 2017. Environmental requirements, p. 254-278. In: R. Socias i Company and T.M. Gradziel (eds.). Almond: botany, production and uses. CABI, Boston, MA.

Anderson, J.L., E.A. Richardson, and C.D. Kesner. 1986. Validation of chill unit and flower bud phenology models for 'Montmorency' sour cherry. Acta Hort. 184:71-78.

Andreini. L., R. Viti, S. Bartolini, D. Ruiz, J. Egea, and J.A. Campoy. 2012. The relationship between xylem differentiation and dormancy evolution in apricot flower buds (*Prunus armeniaca* L.): the influence of environmental conditions in two Mediterranean areas. Trees 26:919-928.

Balandier, P., M. Bonhomme, R. Rageau, F. Capitan and E. Parisot. 1993. Leaf bud endodormancy release in peach trees: evaluation of temperature models in temperate and tropical climates. Agr. Forest Meteorol. 67:95-113.

Batlle, I., F. Dicenta, R. Socias i Company, T.M. Gradziel, M. Wirthensohn, and F.J. Vargas. 2017. Classical genetics and breeding, p. 111-148. In: R. Socias i Company and T.M. Gradziel (eds.). Almonds: botany, production and uses. CABI, Boston, MA.

California Department of Food and Agriculture. 2019. 2018 California almond acreage report. CDFA, Sacramento, CA, USA.

Campoy, J.A., D. Ruiz, and J. Egea. 2010. Effects of shading and thidiazuron + oil treatment on dormancy breaking, blooming and fruit set in apricot in a warm-winter climate. Sci. Hort. 125:203-210.

Campoy, J.A., D. Ruiz, and J. Egea. 2011a. Dormancy in temperate fruit trees in a global warming context: A review. Sci. Hort. 130:357-372.

Campoy, J.A., D. Ruiz, N. Cook, L. Allderman, and J. Egea. 2011b. High temperatures and time to budbreak in low chill apricot 'Palsteyn'. Towards a better understanding of chill and heat requirements fulfilment. Sci. Hort. 129:649-655.

Campoy, J.A., D. Ruiz, N. Cook, L. Allderman, and J. Egea. 2011c. Clinal variation of dormancy progression in apricot. S. Afr. J. Bot. 77:618-630.

Cape Almonds. 2017. Prospectus: Independence cultivar. ZZ2, Mooketsi, South Africa.

Cook, N.C. 2010. Final report: Development and testing of a new chilling model for low chill conditions. Hortgro, Paarl, South Africa.

Cook, N.C. and G. Jacobs. 2000. Progression of aplle (*Malus x domestica* Borkh.) bud dormancy in two mild winter climates. J. Hortic. Sci. Biotechnol. 75:233-236.

Cook, N.C., F.J. Calitz, L.A. Allderman, W.J. Steyn and E.D. Louw. 2017. Diverse patterns in dormancy progression of apples buds under variable winter conditions. Sci. Hort. 226:307-315.

Coville, F.A. 1920. The influence of cold in stimulating the growth of plants. J. Agric. Res. 20:151-160.

Erez, A. 2000. Bud dormancy; phenomenon, problems and solutions in the tropics and subtropics, p. 17-48. In: A. Erez (ed.). Temperate fruit crops in warm climates. Kluwer Academic Publishers, The Netherlands.

Erez, A. and G.A. Couvillon. 1987. Characterization of the influence of moderate temperatures on rest completion in peach. J. Amer. Soc. Hort. Sci. 112:677-680.

Faust, M., A. Erez, L.J. Rowland, S.Y. Wang, and H.A. Norman. 1997. Bud dormancy in perennial fruit trees: physiological basis for dormancy induction, maintenance, and release. HortScience 32:623–629.

Fishman, S., A. Erez., and G.A. Couvillon. 1987a. The temperature-dependence of dormancy breaking in plants – mathematical analysis of a 2-step model involving a cooperative transition. J. Theor. Biol. 124:473-483.

Fishman, S., A. Erez, and G.A. Couvillon. 1987b. The temperature-dependence of dormancy breaking in plants – computer stimulation of processes studied under controlled temperatures. J. Theor. Biol. 126:309-321.

Gilreath, P.R. and D.W. Buchanan. 1981. Rest prediction model for low-chilling 'Sungold' nectarine. J. Amer. Soc. Hort. Sci. 106:426-429.

Gradziel, T.M., R. Curtis, and R. Socias i Company. 2017. Production and growing regions, p. 70-86. In: R. Socias i Company and T.M. Gradziel (eds.). Almonds: botany, production and uses. CABI, Boston, MA.

Guirriero, R., S.E.P. Indiogine, and G. Scalabrelli. 1985. The effect of cyclic and constant temperatures in fulfilling the chilling requirements of two apricot cultivars. Acta Hort. 192:41-48.

Heide, O.M. 2008. Interaction of photoperiod and temperature in the control of growth and dormancy of *Prunus* species. Sci. Hort. 115:309-314.

Kapp, C.J. 2008. Manipulation of the chilling requirement of sweet cherry trees. Master's Thesis, Faculty of AgriScience, Univ. Stellenbosch, Stellenbosch, South Africa. Date last accessed: 10 December 2020. http://library.sun.ac.za/en-za/Pages/Home.aspx

Lang, G.A., J.D. Early, G.C. Martin, R.L. Darnell. 1987. Endodormancy, paradormancy, and ecodormancy—physiological terminology and classification for dormancy research. HortScience 22:371–377.

Linsley-Noakes, G.C., G. Matthee, and P. Allan. 1994. Modification of rest completion prediction models for improved accuracy in South African stone fruit orchards. J. S. Afr.

Soc. Hort. Sci. 4:13-15.

Linsley-Noakes, G.C., M. Louw, and P. Allen. 1995. Estimating Daily Positive Utah Chill Units using daily minimum and maximum temperatures. J. S. Afr. Soc. Hort. Sci. 5:19-23.

Luedeling, E. 2012. Climate change impacts on winter chill for temperate fruit and nut production: A review. Sci. Hort. 144:218-229.

Luedeling, E. and P.H. Brown. 2011. A global analysis of the comparability of winter chill models for fruit and nut trees. Int. J. Biometeorol. 55:411-421.

Luedeling, E., M. Zhang, and E.H. Girvetz. 2009. Climatic changes leading to declining winter chill for fruit and nut trees in California during 1950-2099. Plos One 4(7):1-9.

Luna, V., H. Reinoso, E. Lorezo, R. Bottini, and G. Abdala. 1991. Dormancy in peach (*Prunus persica* L.) flower buds. II. Comparative morphology and phenology in floral and vegetative buds, and the effect of chilling and gibberellin A_3. Trees 5:244-246.

Navorsingsinstituut vir Vrugte en Vrugtegnologie. 1985. Inligtingsbulletin nommer 545: Amandelverbouing. NIVV, Stellenbosch, South Africa.

Prudencio, A.S., P. Martínez-Gómez, and F. Dicenta. 2018. Evaluation of breaking dormancy, flowering and productivity of extra-late and ultra-late flowering almond cultivars during cold and warm seasons in South-East of Spain. Sci. Hort. 235:39-46.

Richardson, E.A., S.D. Seeley, and D.R. Walker. 1974. A model for estimating the completion of rest for ‘Redhaven’ and ‘Elberta’ peach trees. HortScience 1:331-332.

Richardson, E.A., S.D. Seeley, D.R. Walker, J.L. Anderson, and G.L. Ashcroft. 1975. Phenoclimatography of spring peach bud development. HortScience 10, 236–237.

Samish, R.M. and S. Lavee. 1962. The chilling requirement of fruit trees. In: Proc. XVI Int. Hort. Cong. 5:372-388.

Saure, M.C. 1985. Dormancy release in deciduous fruit trees. Hortic. Rev. 7:239-300.

Shaltout, A.D. and C.R. Unrath. 1983. Rest completion prediction model for ‘Starkrimson Delicious’ apples. J. Amer. Soc. Hort. Sci. 108:957-961.

Sparks, D. 1993. Chilling and heating model for pecan Budbreak. J. Amer. Soc. Hort. Sci.

118(1):29-35.

Strydom, D.K., G.E. Honeyborne, and G.J. Eksteen. 1971. Delayed foliation of pome- and stonefruit. Deciduous Fruit Grower. 21:126-129.

Tanino, K.K., L. Kalcsits, S. Silim, E. Kendall, and G.R. Gray. 2010. Temperature-driven plasticity in growth cessation and dormancy development in deciduous woody plants: a working hypothesis suggesting how molecular and cellular function is affected by temperature during dormancy induction. Plant. Mol. Biol. 73:49-65.

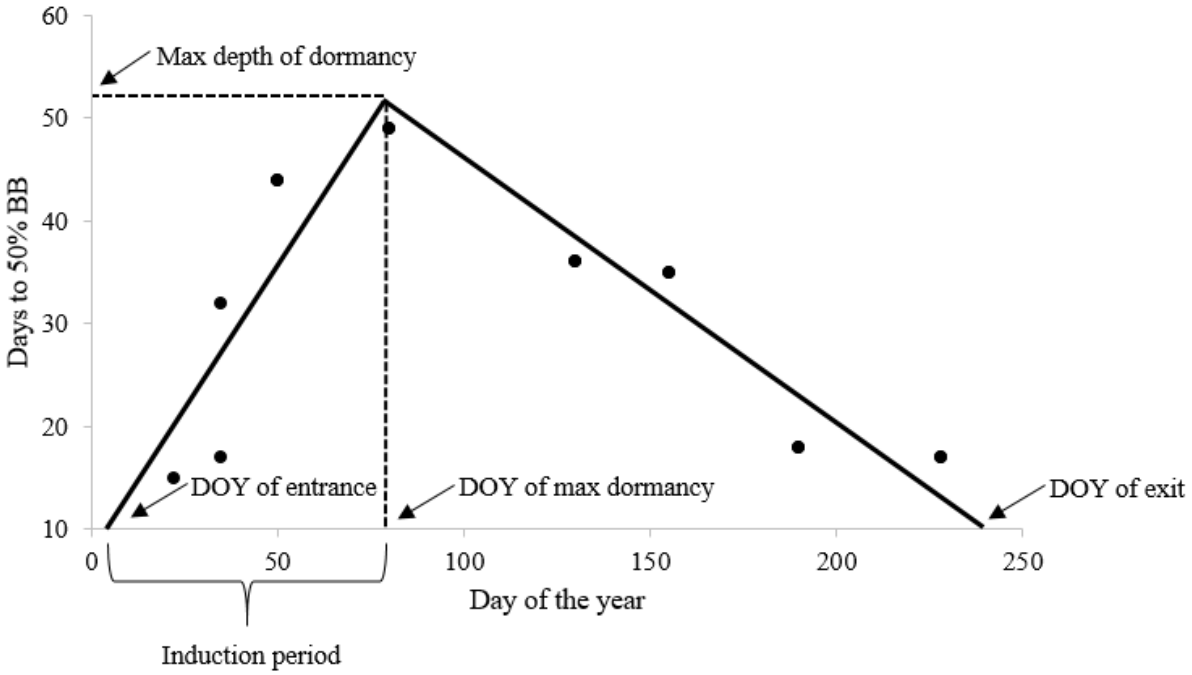


Fig. 1. The two linear joint line model used to fit the scatterplot signifying a typical progression of dormancy indicating the five parameters analysed, namely the DOY of dormancy entrance; the induction period; when maximum dormancy was reached; depth of dormancy; and the DOY of dormancy release. Adapted from Cook et al. (2017). BB = bud break; DOY = day of year

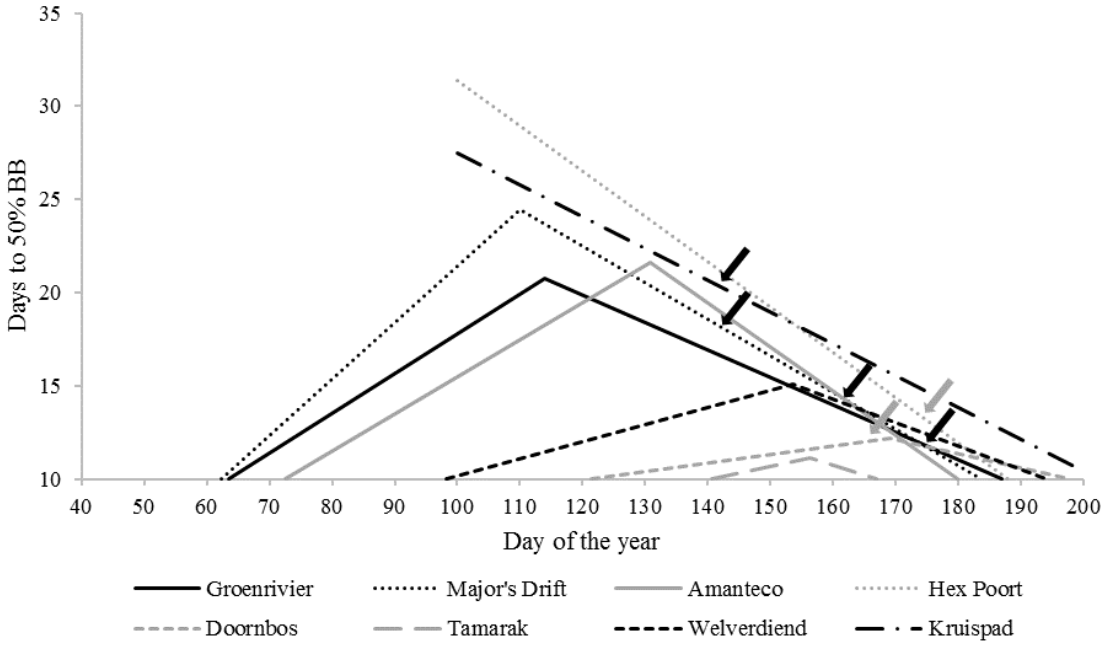


Fig. 2. Two linear joint line models fitted for the mean dormancy progression scatterplot of each orchard during the 2019 season. Arrows indicate the day of the year when each 'Independence' almond orchard started accumulating chill (according to the Utah model). BB = bud break

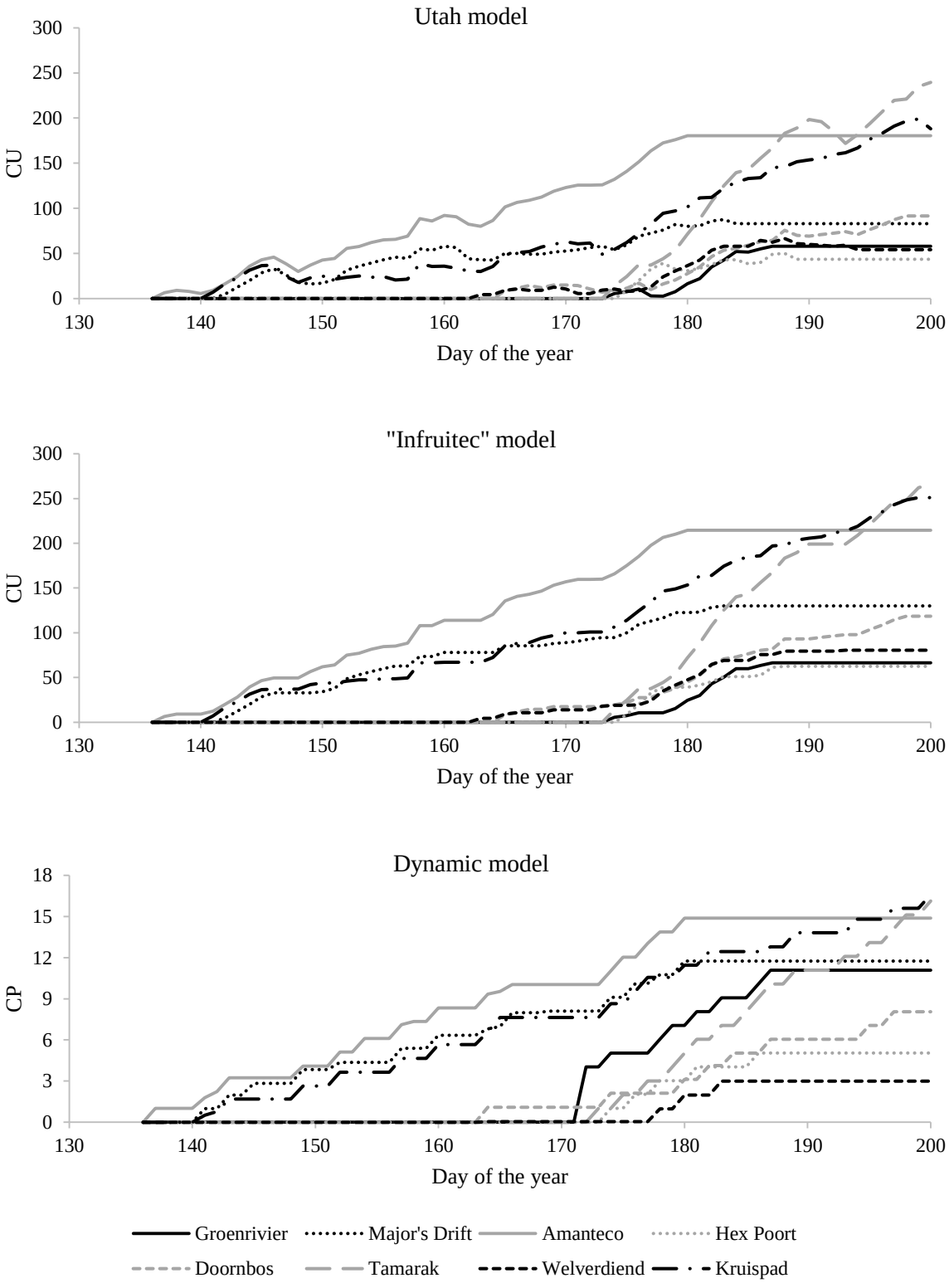


Fig. 3. Chill accumulation according to the Utah, "Infruitec" and Dynamic models during the respective endodormancy periods for each of the eight commercial 'Independence' almond orchards sampled during the 2019 season. CU = chill units. CP = chill portions

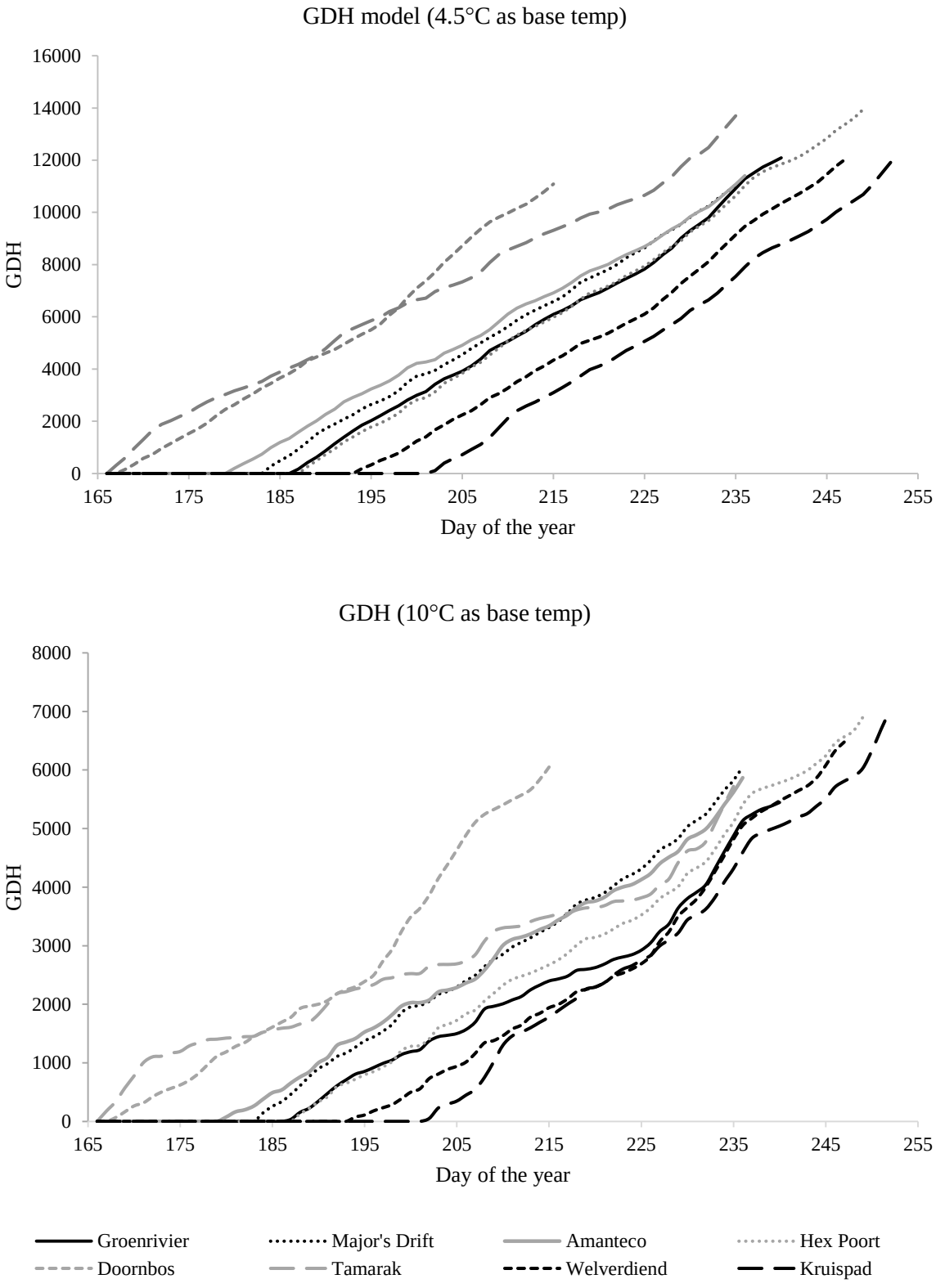


Fig. 4. Heat accumulation according to the Growing Degree Hour (GDH) model with a base temperature of both 4.5°C and 10°C during the respective ecodormancy periods for each of the eight commercial 'Independence' almond orchards sampled during the 2019 season.

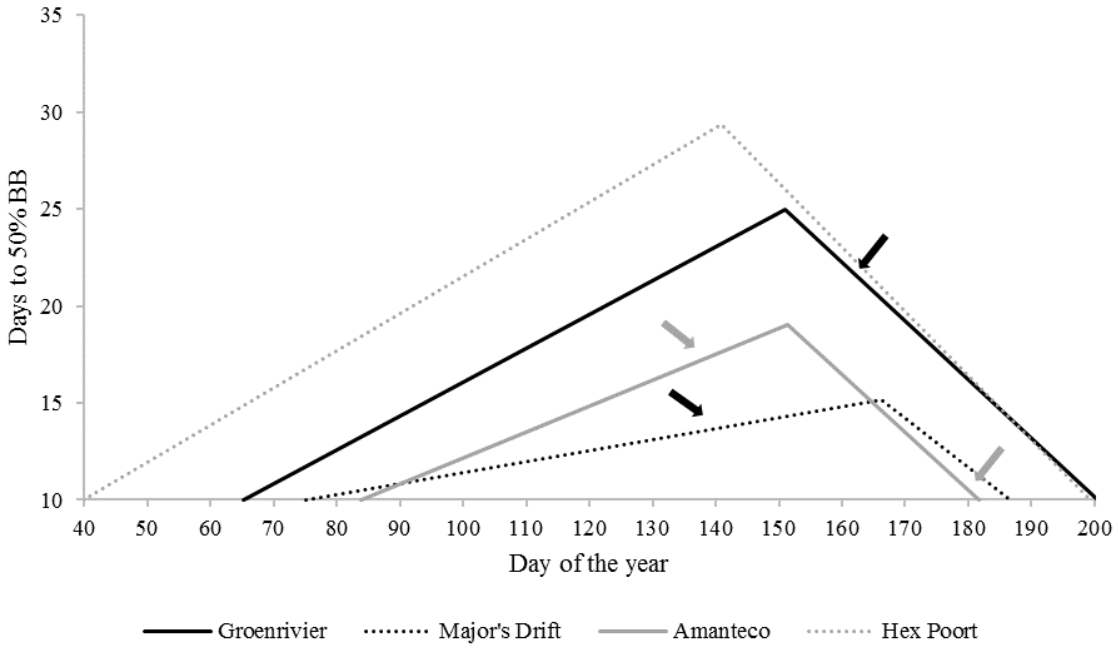


Fig. 5. Two linear joint line models fitted for the mean dormancy progression scatterplot for each 'Independence' almond orchard during the 2020 season. Arrows indicate the day of the year when each orchard started accumulating chill (according to the Utah model). BB = bud break

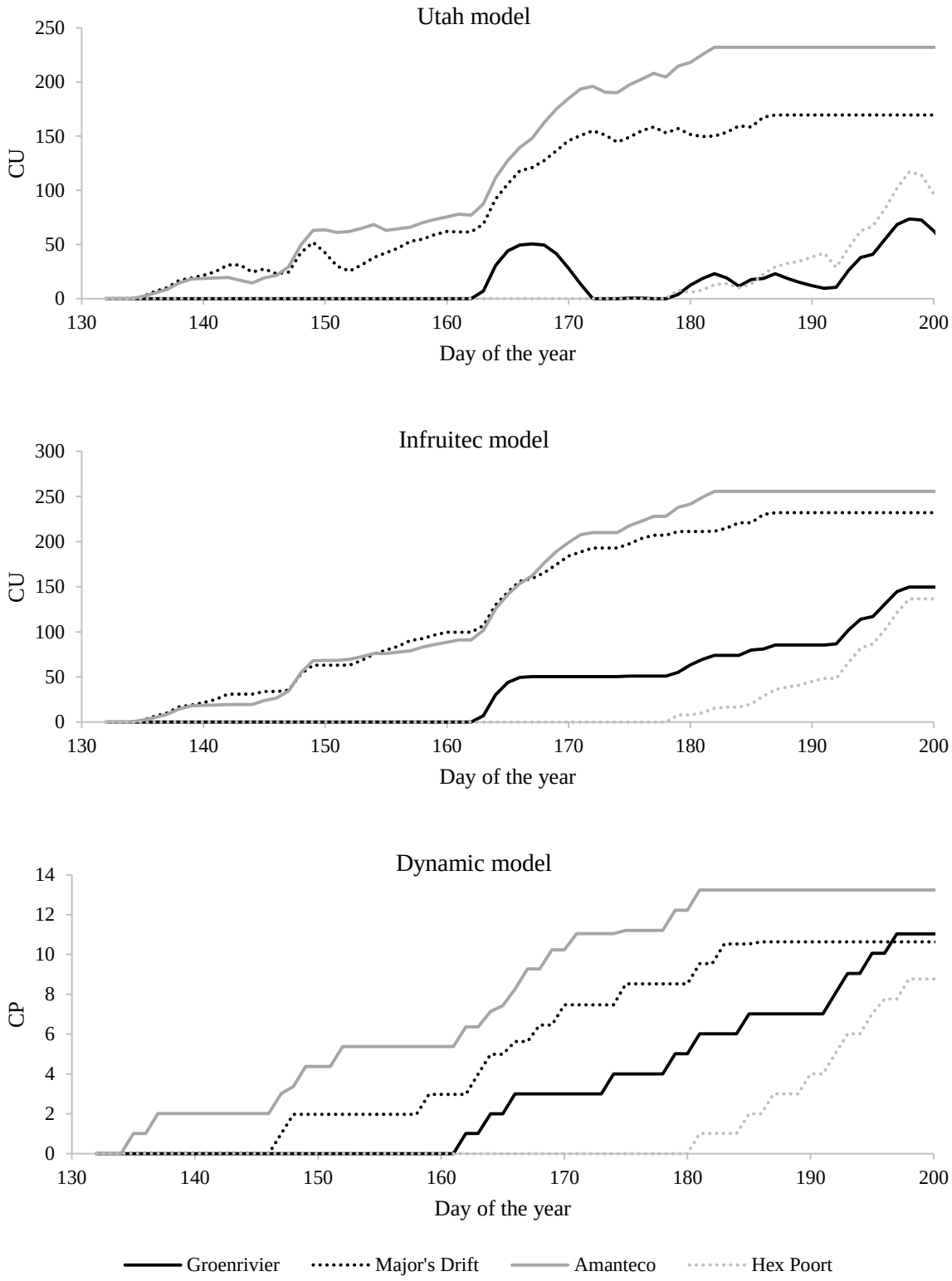


Fig. 6. Chill accumulation according to the Utah, Infruitec and Dynamic models during the respective endodormancy periods for each of the four commercial 'Independence' almond orchards sampled during the 2020 season. CU = chill units. CP = chill portions

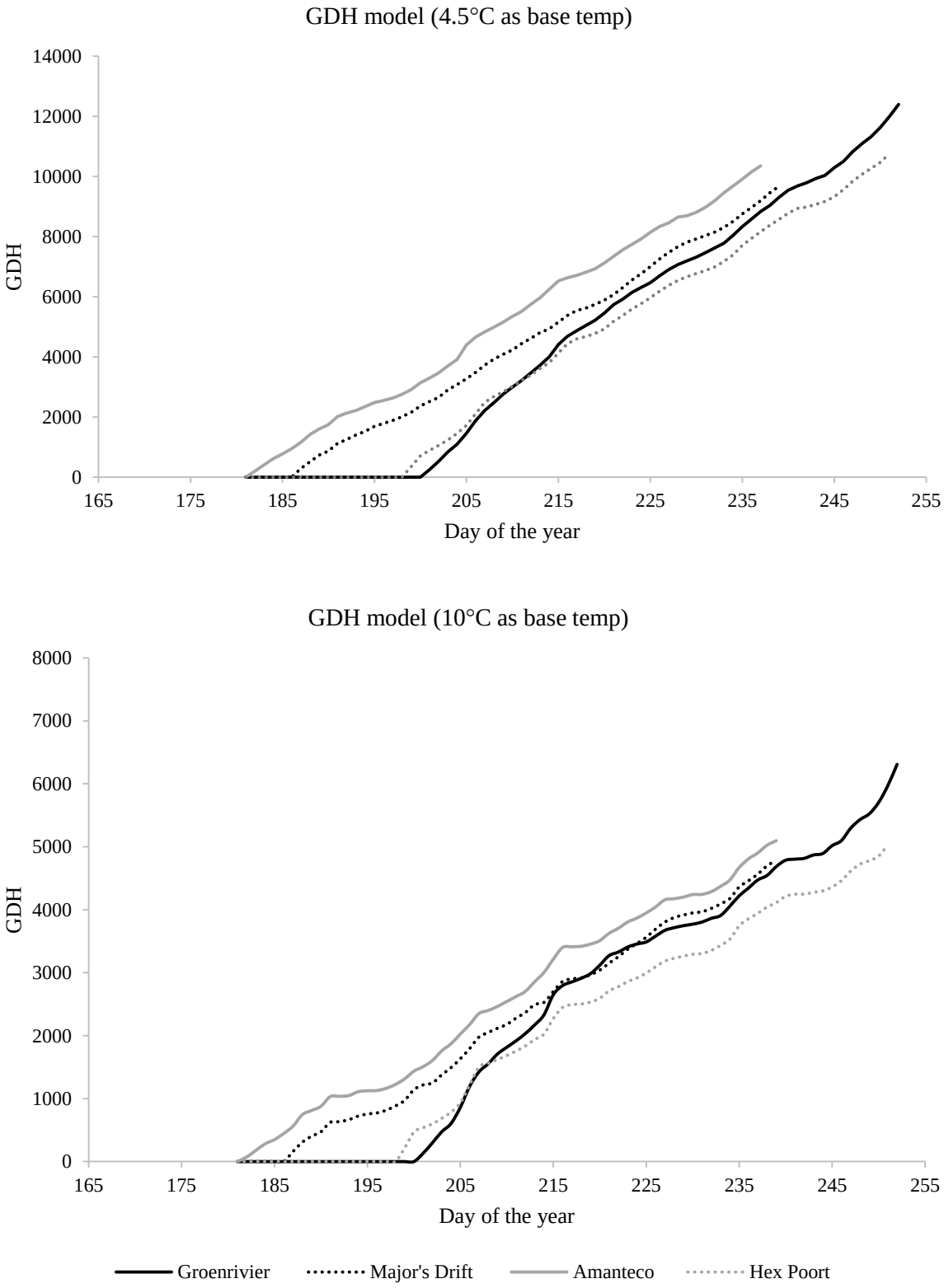


Fig. 7. Heat accumulation according to the Growing Degree Hour (GDH) model with a base temperature of both 4.5°C and 10°C during the respective ecodormancy periods for each of the four commercial 'Independence' almond orchards sampled during the 2020 season.

Table 1: The eight Western Cape 'Independence' orchard locations, including planting dates and plant distance.

Farm	Region	Coordinates	Altitude (m.a.s.l.)	Planting Date	Planting distance
Groenrivier*	Riebeek West	33°20'45.3"S 18°51'52.2"E	193	2016	6 x 4 m
Major's Drift*	Robertson	33°50'17.0"S 19°54'06.0"E	150	2016	6 x 5 m
Amanteco*	Montagu	33°43'22.6"S 20°30'43.9"E	531	2016	6 x 5 m
Hex Poort*	Worcester	33°35'00.4"S 19°29'57.1"E	346	2016	6 x 4 m
Doornbos	Clanwilliam	31°59'31.0"S 19°16'04.3"E	176	2016	6 x 4 m
Tamarak	Piketberg	32°48'53.9"S 18°39'04.1"E	580	2016	7 x 4 m
Welverdiend	Vredendal	31°37'50.0"S 18°26'32.9"E	33	2017	6 x 3.5 m
Kruispad	Oudtshoorn	33°45'18.7"S 22°05'49.7"E	477	2017	6 x 4 m

*The four farms utilized during the 2020 season's trial

Table 2. Dormancy progression for the eight 'Independence' almond orchards during the 2019 season. Entrance = DOY that orchard entered dormancy; Induction period = number of days between entering and reaching maximum depth of dormancy; Max dormancy level = number of days to 50% BB when at maximum dormancy; Exit = DOY that orchard exited from dormancy. Means of each parameter that do not have similar letters differ significantly. ns = no significant difference. DOY = day of the year.

Farm	Region	Entrance (DOY)	Induction period (days)	DOY of max dormancy	Max dormancy level (days)	Exit (DOY)	Endodormancy period (days)	Ecodormancy period (days)	DOY of full bloom
Groenrivier	Riebeek West	63.6 c	50.5 a	114.0 c	20.8 ab	187.0 abc	14.0 c	53.0 ns	240
Major's Drift	Robertson	62.4 c	47.6 a	110.0 c	24.4 a	183.5 bc	42.5 b	52.5	236
Amanteco	Montagu	72.6 c	58.3 a	130.8 bc	21.6 ab	179.9 cd	43.9 b	56.1	236
Hex Poort	Worcester	---*	---*	---*	---*	187.5 abc	13.5 c	61.5	249
Doornbos	Clanwilliam	121.5 a	47.5 a	169.0 a	12.2 c	197.8 ab	33.8 b	47.2	245
Tamarak	Piketberg	140.5 a	15.9 b	156.4 ab	11.1 c	166.8 d	1.4 c	68.2	235
Welverdiend	Vredendal	98.3 b	55.3 a	153.6 ab	15.1 bc	193.9 abc	31.9 b	53.1	247
Kruispad	Oudtshoorn	---*	---*	---*	---*	202.3 a	62.3 a	49.7	252
Significance level		***<0.0001***	***0.0183***	***0.0038***	***0.0090***	***0.0067***	***<0.0001***	*0.1988*	
LSD 5%		*22.20*	*22.79*	*29.33*	*7.40*	*15.90*	*13.26*	-	

*Unable to calculate these values

Table 3. The chill accumulation according to the Utah, "Infruitec" and Dynamic models, as well as the heat accumulation according to the Growing Degree Hours (GDH) model for 'Independence' almond trees during the 2019 season. CU = chill units. CP = chill portions

Farm	Chill accumulation*			Heat accumulation	
	Utah model (CU)	Infruitec model (CU)	Dynamic model (CP)	GDH model (base temp 4.5°C)	GDH model (base temp 10°C)
Groenrivier	49.3 bc	62.3 cde	5.8 c	12028 ns	5374 ns
Major's Drift	81.3 bc	128.7 b	10.5 b	11493	6114
Amanteco	182.2 a	216.2 a	13.9 ab	11397	5859
Hex Poort	44.8 bc	58.7 de	3.7 cd	13999	6907
Doornbos	94.3 b	120.3 bc	6.9 c	11158	6176
Tamarak	17.2 c	17.2 e	0.4 d	13453	5467
Welverdiend	83.3 bc	107.2 bcd	4.0 c	12287	6010
Kruispad	105.8 b	268.0 a	16.7 a	11675	6821
Significance level	***0.0079***	***<0.0001***	***<0.0001***	*0.6060*	*0.6720*
LSD 5%	*72.52*	*58.16*	*3.45*	-	-

*To compare chill accumulation among different models, the start date of chill accumulation for all three models was selected as the date at which the Utah model no longer negated chill units to zero

Table 4. Dormancy progression for the eight 'Independence' almond orchards during the 2020 season. Entrance = DOY that orchard entered dormancy; Induction period = number of days between entering and reaching maximum depth of dormancy; Max dormancy level = number of days to 50% BB when at maximum dormancy; Exit = DOY that orchard exited from dormancy. Means of each parameter that do not have similar letters differ significantly. ns = no significant difference. DOY = day of the year

Farm	Region	Entrance (DOY)	Induction period (days)	DOY of max dormancy	Max dormancy level (days)	Exit (DOY)	Endodormancy period (days)	Ecodormancy period (days)	DOY of full bloom
Groenrivier	Riebeek West	65.2 ns	85.8 ns	151.0 ab	25.0 ns	200.6 a	38.6 b	51.4 ns	252
Major's Drift	Robertson	75.2	91.1	166.2 a	15.2	186.7 bc	52.7 a	52.3	239
Amanteco	Montagu	83.8	67.6	151.4 ab	19.0	181.7 c	47.7 ab	57.3	239
Hex Poort	Worcester	39.8	101.0	140.8 b	29.3	199.3 ab	21.3 c	51.7	251
Significance level		*0.0838*	*0.0811*	***0.0331***	*0.2659*	***0.0207***	***0.0019***	*0.6878*	
LSD 5%		-	-	*15.51*	-	*12.55*	*12.55*	-	

Table 5. The chill accumulation according to the Utah, "Infruitec" and Dynamic models, as well as the heat accumulation according to the Growing Degree Hours (GDH) model for 'Independence' almond trees during the 2020 season. CU = chill units. CP = chill portions

Farm	Chill accumulation*			Heat accumulation	
	Utah model (CU)	Infruitec model (CU)	Dynamic model (CP)	GDH model (base temp 4.5°C)	GDH model (base temp 10°C)
Groenrivier	49.3 d	143.3 b	10.7 ns	12561 ns	6410 ns
Major's Drift	163.7 c	227.5 a	10.2	9773	4830
Amanteco	228.2 b	250.7 a	12.8	10414	5115
Hex Poort	346.5 a	269.0 a	12.8	10620	4900
Significance level	***<0.0001***	***0.0034***	*0.1125*	*0.1816*	*0.0969*
LSD 5%	*50.04*	*54.97*	-	-	-

*To compare chill accumulation among different models, the start date of chill accumulation for all three models was selected as the date at which the Utah model no longer negated chill units to zero.

PAPER 2: The influence of artificial rest breaking agents on vegetative and reproductive growth of 'Independence' almond trees

Additional index words. Bud break, *Prunus amygdalus* Batsch, bearing position, growth index, fruit set, quality

Abstract.

Insufficient winter chill causes delayed and/or reduced bud break due to incomplete dormancy release, which can compromise yield and tree architecture in deciduous fruit trees. Due to marginal winter conditions, the use of chemical rest breaking agents (RBAs) to overcome dormancy and address challenges associated with insufficient winter chill has become standard practice in the South African pome and stone fruit industry. The purpose of this study was to determine the efficacy of seven rest breaking treatments on 'Independence' almond trees to improve bud break, flowering patterns, and potential bearing positions for consecutive seasons in two commercial orchards in the Western Cape. The effect on fruit set, yield and post-harvest quality were also considered. The seven treatments consisted of 0.5% hydrogen cyanamide (HC); 2% mineral oil; a mixture of 0.5% HC and 2% mineral oil; 2.5% thidiazuron (TDZ); 50 g·L^{-1} KNO_3; a mixture of 50 g·L^{-1} KNO_3 and 2% oil; and an untreated control. The rest breaking treatments did not have an effect on reproductive bud break, fruit set, yield efficiency or any of the post-harvest quality parameters. Rest breaking treatments, 0.5% HC + 2% Oil, did however accelerate vegetative bud break, causing an overlap between reproductive and vegetative growth and development. Rest breaking treatments containing oil increased the number of new bearing positions by enhancing new spur formation. However, the increase in spur production compromised further vegetative growth in trees subject to stress-related conditions, such as water shortage and competition for available nutrients.

Most of the deciduous fruit growing regions in South Africa are characterized by marginal winter chilling of less than 1000 Richardson Chilling Units (CU) each year (Linsley-Noakes, 1994; Costa et al., 2004). Inadequate winter chilling results in incomplete dormancy release in deciduous fruit trees (Erez, 2000). This can affect tree architecture, cause poor and unsynchronized bud break, as well as protracted bloom, which can hinder successful cross-pollination if flowering times do not overlap (Costa et al., 2004; Erez, 1987). Lack of winter chilling can thus compromise the yield, fruit size and tree architecture, while complicating other orchard management practices such as fruit thinning and harvesting. The chilling requirements vary among individual buds on a tree. Reproductive buds have a lower chilling requirement compared to vegetative buds, whereas the chill requirement for lateral buds is higher than terminal buds (Saure, 1985). This typically leads to smaller flower size, flower drop or embryo abortion, resulting in excessive early fruit drop, while typically causing rosette formation in vegetative buds (Erez, 2000).

In the South African pome (Costa et al., 2004) and stone (Sheard et al., 2009) fruit industry, chemical rest breaking agents (RBAs) have become standard practice to increase and synchronize bud break, as well as address problems associated with delayed foliation. Various chemical RBAs break bud dormancy, such as oils, dinitro-ortho-cresol (DNOC), cyanamide, thiourea, growth regulators and potassium nitrate (Erez, 1987; Lloyed and Firth, 1993; Erez, 2000; Costa et al., 2004), as well as combinations of the aforementioned chemicals. However, only a few are commercially acceptable, having met the required characteristics such as low cost, strong results and minimum toxic effect on humans and plants (Erez, 2000). Oil treatments, and any treatment combinations that include oil, deprive buds of oxygen, causing anaerobic conditions and break dormancy due to the production of ethanol (Erez et al., 1980) at low oxygen levels. Treatments with hydrogen cyanamide (HC) increase respiration by reducing the catalase activity, while peroxidase levels remain constant (Shulman et al., 1986). This increases oxidation, which ultimately leads to the breaking of dormancy in treated plants (Shulman et al., 1986). Other treatments that interfere with aerobic respiration, such as thidiazuron (TDZ), successfully break bud dormancy by increasing promoters of bud break, such as cytokinins and gibberellins (Faust et al., 1991). When potassium nitrate (KNO_3) is applied during low oxygen conditions, nitrate reductase reduces the nitrate (NO_3^-) to nitrite (NO_2^-) and eventually to nitric oxide (NO) (Yamasaki et al., 1999). Nitric oxide is a reactive nitrogen species that competes for oxygen by inhibiting cytochrome oxidase (Brown and Borutaite, 2002), while increasing the content of reactive oxygen species by inhibiting catalase

activity (Brown, 1995). However, no single chemical RBA, or combination of RBAs can fully substitute natural chill accumulation. The exposure to partial chilling is necessary for normal bud break to proceed (Erez, 2000).

Vegetative growth and reproductive bud development both regulate tree growth and bearing habits (Sarvisé and Socias i Company, 2004). In *Prunus* species, flower buds develop prior to vegetative buds (Alonso, 2017). Once the flower initiation is established in the meristem, reproductive bud development forms the different floral organs. This developmental process takes place relatively slowly during the winter, followed by a sudden acceleration a few weeks prior to blooming (Socias i Company et al., 2017). Reproductive bud dormancy, winter survival rate and the ability to develop normal floral organs in the subsequent spring determine the yield of almond (Szalay, 2006). For most of the chemical RBAs, the reproductive buds are the most sensitive tree organs to phytotoxicity and loss of flowers (Erez, 2000). A tree's vegetative growth is subject to both environmental conditions, such as water status, and intrinsic features giving each almond cultivar a unique growth habit (Socias i Company et al., 2017). Vegetative growth takes place rapidly after vegetative bud break, which is essential for the establishment of new bearing positions and carbohydrate reserves necessary for future yields (Doll, 2017). Chemical RBAs that increase vegetative bud break, increases spur production for the following season (Erez, 2000). According to the author, this phenomenon could have a favourable effect on the yield for several years by increasing the potential bearing positions. The seed (kernel) is the commercial part of the almond; therefore, pollination and subsequent fertilization of the ovule is essential to produce a crop. Due to the small size of almond kernels, a large number of fruit needs to be produced to ensure a commercially viable almond yield (Godini, 2002). This is achieved by ensuring a high flower density, as well as the efficient pollination and fertilization of as many flowers possible (Ortega et al., 2004; Socias i Company et al., 2017).

The American based company, Zaiger's Inc. Genetics released the self-compatible almond cultivar, Independence, in the USA in 2008, after which cultivated hectares expanded substantially (California Department of Food and Agriculture, 2019). 'Independence' almond trees are well suited for warmer growing regions, such as the Western Cape, South Africa, due to its proposed lower chill requirement of 400 CU (Utah model) and 23.7 chill portions (Dynamic model) (Cape Almonds, 2017). Since receiving the exclusive master-license to cultivate 'Independence' in Southern Africa, ZZ2 (Cape Almonds), in partnership with several sub-licensee growers, have planted more than 500 ha in various micro-climatic regions

throughout the Western Cape (personal communication, Cape Almonds). The later flowering and early harvesting window of this cultivar contributes to its popularity with producers, due to inherit lower risk for late winter frost and early winter rain (Cape Almonds, 2017).

The United States is the largest almond producing country, supplying more than 70% of the world's almonds (Gradziel et al., 2017). California is characterized by intermediate winter chill of 700 – 2000 Richardson CU per year (Luedeling and Brown, 2011), which makes almond a well-suited crop due to characteristics such as low chilling requirements and early flowering (Alonso, 2017). Chemical RBAs are therefore not used in these regions to overcome bud dormancy. Australia, being the second largest almond producer and contributing 7% to the global almond production (Australian Almonds, 2020), also does not make use of artificial RBAs to break dormancy (personal communication, Zelmari Coetzee, Agriculture Victoria). In the South African context however, chemical RBAs have become standard commercial practice for deciduous fruit crops (Strydom et al., 1971; Costa et al., 2004; Sheard et al., 2009). Almonds are still a novel crop in South Africa, contributing very little to the global almond supply in the past (Cape Almonds, 2017).

Scientific data on the effects of artificial RBAs on almond are scarce. Therefore, the objective of this study was to determine the efficacy of seven rest breaking treatments on 'Independence' almond trees to improve bud break, flowering patterns and possible new bearing positions on two commercial farms in the Western Cape. The increase in possible bearing positions for the subsequent season was also investigated, as well as the effect on fruit set.

Materials and Methods

Plant material and site description. Rest breaking trials were conducted over two consecutive seasons on three and four-year-old 'Independence' almond orchards on two commercial farms in the Western Cape, South-Africa, viz. Groenrivier (33°20'45.3"S 18°51'52.2"E; 193 m.a.s.l.) in Riebeek West and Hex Poort (33°35'00.4"S 19°29'57.1"E; 346 m.a.s.l) in Worcester. Both orchards on 'Viking' rootstocks were planted at a spacing of 6 x 4 m in 2017. Orchards received standard commercial cultivation practices and produced their first commercial crop in February 2019. The orchard at the Worcester trial site had to compromise their irrigation during March and April 2020, just after harvest, due to water

shortage. Some of the trees in this trial site therefore received less irrigation water during a critical time, which could influence the growth and yield in the subsequent spring and summer (Doll, 2017). Temperature data were obtained for each orchard by placing Tinytag Plus 2 data loggers (Gemini Data Loggers, UK) in each study site recording hourly temperature for the entire duration of the trial.

Trial lay-out and treatment application. Randomised complete block designs were used at both trial sites, with seven rest breaking treatments and ten, single tree replicates (total of 70 trees per trial site). Six rest breaking treatments, together with an untreated control, were compiled out of four different products, viz. HC (Dormex®), oil (Opron®), TDZ (Lift®) and KNO_3, as summarized in Table 1. None of the big role-playing countries that produce almonds make use of rest breaking treatments to break dormancy, hence, there is no industry standard. RBAs were therefore applied according to other stone fruit industry standards (Glozer and Coates, 2006; Sheard et al., 2009; Niederholzen and Glozer, 2019).

To ensure optimal coverage of the trees, the treatments were applied using a motorised knapsack sprayer (STIHL, Pietermaritzburg, South Africa) at approximately 1000 $L \cdot ha^{-1}$ (1L per tree). To prevent any possible cross contamination due to spray drift, at least one untreated tree was left between the treated trees in a row and one untreated row between the treated rows. For the 2019/2020 season, all the treatments were applied in both orchards on 1 August 2019 after bud swell, Stage B, according to the scale developed by Felipe in 1977 (Prudencio et al., 2018) had occurred as depicted in Appendix C. The treatments were applied in conditions of clear skies, no/very low wind speed and temperatures between 14-18 ℃. For the 2020/2021 season, the treatments were applied in both orchards on 28 July 2020. Weather conditions were like the previous year, with temperatures ranging between 12 and 14 ℃.

Data collection. To determine bud break in the 2019/2020 season, two scaffold branches were randomly selected and tagged on each tree during the dormant phase. One of the branches was a simple structure, consisting of a single, one-year-old shoot on two-year-old wood, and one complex structure, which included two or more one-year-old shoots, including sylleptic growth, on two-year-old wood. All the shoots on each scaffold branch were categorised according to shoot type, viz. shoots with sylleptic growth, single long shoots (>20 cm), short shoots (3 – 20 cm) and spurs (<3 cm). The length of each shoot was measured, and the number of dormant buds were counted, resulting in a detailed description of two branches on each tree. Two weeks after RBAs were applied, the monitoring of bud break started by

visiting each orchard twice a week. Bud break was recorded when the calyx was visible for reproductive buds and at green tip stage for vegetative buds, as shown in Appendix C. Vegetative- and reproductive bud break on each of the tagged branches were counted at every visit for approximately four weeks (until 18 September 2019) until no further bud break occurred. An additional branch was randomly selected and tagged on each tree and used to calculate fruit set percentage. The total number of flowers on each tagged branch was counted at full bloom (80% flowering). Six weeks after full bloom, the number of fruitlets present on the tagged branches was counted. Fruit set was determined by the total number of almonds divided by the total number of flowers on the three tagged branches and was expressed as percentage fruitlets per tree.

For the 2020/2021 season, bud break was monitored by tagging five long shoot, five short shoots and five spurs on each of the treated trees at the Groenrivier site. At the Hex Poort trial site, hardly any long shoots were present, therefore, only five short shoots and five spurs were tagged. Bud break monitoring started a week after treatment application and commenced in a similar way to the previous year. Monitoring started on 3 August 2020 and each orchard was visited at least once a week. The number of vegetative- and reproductive buds sprouted on each of the tagged branches were counted at every visit for approximately nine weeks (until 9 October 2020) until no further bud break occurred. At approximately six weeks after full bloom, the number of fruitlets that set on the branches were counted and fruit set was determined like the 2019/2020 season.

Harvesting took place on 30 and 31 January 2020 at Groenrivier, and 11 and 12 February 2020 at Hex Poort, as per commercial practice. Each tree was harvested individually and the nuts per tree kept separate for the duration of the trial. After harvest, the fruit were dried for three to four days until the kernels reached a moisture content of 6%. The moisture content of the kernels was measured using a digital moisture analyser (Wincom model XY – 100MW – T, Jiangsu, China). The dry weight of the yield (kg) per tree was recordered and the hulls were removed. After hulling, the in-shell weight of the yield (kg) per tree was recorded and the kernels were removed from the shells. After shelling, the kernel weight of yield (kg) per tree was recorded and the kernel percentage per tree was determined by dividing the kernel weight by the in-shell weight of each tree. The dry weight, in-shell weight and kernel weight per tree were all recorded using an UWE check weighing scale (Elec-Checking Scale model NBK-30, Cape Town, South Africa). Yield efficiency ($g \cdot cm^{-2}$) was calculated from the total

yield per tree and trunk cross-sectional area (TCSA), measured after harvest 7 cm above the graft union.

To determine the post-harvest quality parameters, a sub sample of 50 kernels per replication per trial site were brought to the laboratory for non-destructive analysis. For all the fruit in the sample, single kernel weight, length, width, and thickness, as well as pellicle colour and roughness were determined. Single kernel weight was determined using a Kern precision balance (model number PLJ 700-3CM, Balingen, Germany), while the size of the kernels was determined using a Mitutoyo ABSOLUTE Digimatic caliper (model number 500-196-30, Tokyo, Japan). A visual scoring of each kernel sample was made to determine the pellicle colour by using the score chart in Fig. 1.1 with values ranging from 1 (very light brown) to 5 (very dark brown). Kernel roughness was scored by comparing the surface texture of each nut to the chart in Fig. 1.2, values ranged from 1 (smooth) to 3 (wrinkled). To determine the percentage defects, a separate sample of 10% of the tree kernel weight (per replication, per trial site) were screened for double and shrivelled kernels (as seen in Fig. 1.3). Data for the 2021 harvest will not be included in this study.

To determine the effect of the treatments on vegetative growth and thus potential new bearing positions, the new growth (vigour) of the consecutive season was measured. Ten one-year-old shoots (five long and five short shoots) were selected on each tree during the dormant period of 2019. All the new growth that developed from these pre-selected (primary) shoots was counted, measured and classified as either secondary shoots with sylleptic growth, long shoots (>20 cm), short shoots (3 - 20 cm) or spurs (>3 cm) after growth cessation on 24 July 2020 for Groenrivier, and 25 July for Hex Poort (Fig. 2). The growth index of each tree was determined by calculating the ratio of the total length (cm) of secondary growth (sum of shoots with sylleptic growth, long and short shoots, as well as spurs) to the total length of the primary shoots. Similarly, growth indexes were determined for each of the four secondary growth categories.

Data handling. The total, vegetative and reproductive bud break for each season was evaluated and used to quantify the efficacy of each RBA, compared to the untreated control. The bud break data were analysed to determine the onset period (number of days to 5% bud break after treatment application), the maximum percentage bud break, and the bud break period (number of days from 5% to the maximum percentage bud break) of vegetative, reproductive and total bud break as the percentage of total dormant buds. An additional

parameter was included for vegetative bud break, namely the percentage vegetative bud break when reproductive bud break was at its maximum (Fig. 3).

The percentage bud break data were calculated as follow:

Vegetative BB = $\frac{number\ of\ vegetative\ bud\ break}{total\ number\ of\ dormant\ buds}\ x\ 100$

Reproductive BB = $\frac{number\ of\ reproductive\ bud\ break}{total\ number\ of\ dormant\ buds}\ x\ 100$

Total BB = $\frac{number\ of\ vegetative + reproductive\ bud\ break}{total\ number\ of\ dormant\ buds}\ x\ 100$

Statistical analysis. Differences amongst the treatments were analysed by using the linear model procedure and when the F-statistic indicated significant differences at a 5% level, Fisher's LSD test was performed as a post hoc test in SAS Enterprise Guide 7.1 (SAS Institute Inc., Cary, North Carolina, USA).

Results

Results from the 2019/2020 season:

Riebeek West. No significant differences were found in the time of bud break onset, maximum percentage bud break or the bud break period of reproductive buds as a percentage of the total number of dormant buds at the Riebeek West orchard between the different treatments and the untreated control (Fig. 4a; Table 2).

Significant differences were found in the onset (p<0.0001), the percentage vegetative bud break at maximum reproductive bud break (p=0.0001), the maximum percentage (p=0.024) and the period (p<0.002) of vegetative bud break (BB) as a percentage of the total dormant buds among the different treatments and the untreated control (Table 2). The HC + Oil and KNO_3 treatments induced the fastest vegetative BB onset, but did not differ significantly from that of Oil, and TDZ. The latter treatments did not differ significantly from HC either. KNO_3 and the control had a significantly delayed vegetative BB onset, compared to the rest of the treatments. For the vegetative bud break at maximum reproductive bud break (BB_{veg}@maxBB_{rep}), HC + Oil and KNO_3 + Oil showed the highest percentage bud break, but did not differ significantly from that of Oil and HC. Oil and HC did not differ significantly

from TDZ or the control, while KNO_3, showing the lowest percentage bud break, did not differ from the control either. For the maximum vegetative bud break, the HC + Oil treatment, again, induced the highest percentage, however, none of the treatments differed significantly from the untreated control, with KNO_3 having the lowest maximum percentage vegetative bud break. The HC + Oil, TDZ and KNO_3 + Oil treatments induced the shortest vegetative bud break period, but did not differ significantly from that of Oil. The Oil treatment did not differ significantly from that of KNO_3, which, in turn, did not differ significantly from that of HC and the untreated control (Fig. 4b).

No significant differences were found in the time of total bud break onset as a percentage of the total number of dormant buds among the various RBAs and the untreated control (Fig. 4c). Significant differences were, however, found in the maximum percentage total bud break (p=0.0074), as well as the bud break period (p=0.0003) (Table 2). All the treatments, as well as the untreated control, had a high maximum percentage of total bud break greater than 95%. The HC + Oil, Oil, TDZ and KNO_3 + Oil had the higher maximum percentage total bud break, but did not differ significantly from that of HC. The KNO_3 treatment and untreated control had the lowest maximum percentage bud break, but did not differ significantly from that of HC. The total bud break period was between 22 and 31 days for the Riebeek West orchard. The HC + Oil resulted in the shortest period, but did not differ significantly from Oil, TDZ and KNO_3 + Oil. These four treatments had a significantly shorter total bud break period compared to HC, KNO_3 and the control, with the control having the longest period. The average fruit set results in the Riebeek West orchard ranged from 36.7 - 42.4% but no significant differences were induced by the RBAs (Table 2).

The yield and post-harvest quality parameters did not differ significantly in dry weight, in-shell weight, kernel weight, percentage kernel, as well as the yield efficiency per tree, between the different treatments and the untreated control (Table 3). There were also no significant differences in the weight and width of individual kernels, as well as the percentage double and shrivelled kernels between the treatments and control (Table 3 and 4). Significant differences were however found in the kernel length (p=0.0268), kernel thickness (p=0.0003), pellicle colour (p<0.0001) and the kernel roughness (p<0.0001), but none of these differences were large enough to be of horticultural importance (Table 3 and 4).

No significant differences were found in growth index for all secondary growth, shoots with sylleptic growth, long and short shoots, amongst the different RBAs and the untreated

control (Table 5). However, the growth index for the secondary spur growth differed significantly among the different treatments and the control (p<0.0001). The growth index ranged from 0.24 – 0.52, with the secondary spur index significantly higher following HC + Oil application, compared to the other treatments. The Oil, TDZ and KNO_3 + Oil treatments did not differ significantly from each other, but had a significantly higher index compared to HC, KNO_3 and the control.

The new vegetative growth results had a significant interaction between RBAs and the primary shoot type for the average number of secondary shoots with sylleptic growth (p=0.0470) formed (Table 6). The control and HC treatment, in combination with the primary long shoots had the highest average number of secondary shoots with sylleptic growth, but did not differ significantly from that of the KNO_3 + Oil treatment in combination with the primary long shoots. The rest of the treatment combinations had significantly fewer secondary shoots with sylleptic growth. However, very few secondary shoots with sylleptic growth were formed overall.

No significant interactions were found between the different RBAs and the primary shoot type in the average number of secondary long and short shoots formed and neither did the rest breaking treatments influence these (Table 7). There were, however, significant differences (p<0.0001) in the average number of secondary long and short shoots formed for the different primary shoot types. The primary long shoots produced a significantly higher average number of secondary long shoots (0.97), compared to the primary short shoots (0.20). Likewise, primary long shoots produced a significantly higher average number of secondary short shoots (10.31), compared to short shoots (3.17).

A significant interaction (p=0.0004) was found between the RBAs and the primary shoot types for the average number of secondary spurs formed (Table 6). The HC + Oil treatment, in combination with primary long shoots had the highest average number of secondary spurs formed (74.1). The Oil, TDZ and KNO_3 + Oil treatments in combination with primary long shoots did not differ significantly from each other. These treatments, in combination with primary long shoots did, however, result in a higher average number of secondary spurs formed, compared to HC, KNO_3 and the control, in combination with primary long shoots. The average number of secondary spurs formed in trees treated with HC + Oil, Oil, TDZ and KNO_3 + Oil treatments, in combination with primary short shoots, did not differ significantly from each other. While the Oil, TDZ, HC, and KNO_3 treatments in combination

with primary short shoots did not differ significantly from the untreated control in combination with primary short shoots (8.7).

Worcester. No significant differences were found in the onset of reproductive bud break, maximum percentage bud break or bud break period as a percentage of the total dormant buds in the Worcester orchard (Fig 5a; Table 8). Significant differences in vegetative bud break as a percentage of the total dormant buds occurred in the onset ($p<0.0001$) and bud break period ($p=0.011$) (Table 8). The TDZ treatment had the shortest onset, but did not differ significantly from HC + Oil, Oil and KNO_3 + Oil. The KNO_3 treatment had the longest onset, but did not differ significantly from the untreated control. KNO_3 had the shortest vegetative bud break period, but did not differ significantly from the control. None of the other treatments differed significantly from the control, except TDZ, with the longest vegetative bud break period. No significant differences were, however, found for the maximum percentage bud break, or the vegetative bud break at maximum reproductive bud break (Fig. 5b).

No significant differences in total bud break as a percentage of the total number of dormant buds occurred in onset, maximum percentage, and period of total bud break, as well as the average percentage fruit set between the various RBAs and the untreated control (Fig. 5c and Table 8). The yield and post-harvest quality parameters did not differ significantly between the different RBAs and the untreated control, except the kernel length ($p=0.0500$) (Table 9) and pellicle colour ($p=0.0404$) (Table 10), but none of these differences were large enough to be of horticultural importance.

The growth index at the site in the Worcester region, differed significantly in all secondary growth ($p=0.0061$), secondary short shoots ($p=0.0070$) and secondary spurs ($p<0.0001$) between the different RBAs and the untreated control (Table 11). In the case of all secondary growth, none of the treatments differed significantly from the control, except for HC, TDZ and HC + Oil, the latter having the lowest growth index of 0.48. Also, in the case of secondary short shoots, HC + Oil had the lowest growth index of 0.26. This did not differ from HC, Oil, TDZ or KNO_3. The KNO_3 + oil treatment had the highest growth index of 0.68, but did not differ significantly from KNO_3 or the untreated control. However, when considering secondary spur growth, HC + Oil had the highest growth index (0.22), but did not differ significantly from TDZ and KNO_3 + Oil. The KNO_3 treatment had the lowest spur growth index (0.06), but did not differ significantly from the control. No significant differences were

found in the secondary shoots with sylleptic growth and long shoot growth indexes between the different RBAs and the untreated control (Table 11).

No significant differences in new vegetative growth in the average number of secondary shoots with sylleptic growth, long and short shoots formed were found among the different RBAs and the untreated control (Table 12). There were also no significant interactions between the rest breaking treatments and the primary shoot types in the average number of secondary shoots with sylleptic growth, long and short shoots formed. However, significant differences were found in the average number of secondary shoots with sylleptic growth (p=0.0001) and short shoots (p<0.0001) formed between the different primary shoot types, but not for the number of secondary long shoots formed (Table 12). The primary long shoots produced a significantly higher number of secondary shoots with sylleptic growth (0.23), compared to the primary short shoots that produced no secondary shoots with sylleptic growth. Likewise, primary long shoots produced a significantly higher average number of secondary short shoots (10.86), compared to the primary short shoots (4.36).

A significant interaction (p=0.0004) was found between the different rest breaking treatments and the primary shoot types in the average number of secondary spurs formed (Table 13). Treatments with a significantly higher number of secondary spurs formed on primary long shoots, did not necessarily have a significantly higher number of secondary spurs on shorts shoots, compared to the other RBAs and the control. The different treatments therefore had a different effect on the number of secondary spurs formed, when different shoot types were considered.

The primary long shoots of the HC + Oil treatment had the highest average number of secondary spurs (27), but did not differ significantly from the number of secondary spurs formed on the primary long shoots of the Oil and KNO_3 + Oil treatments. The number of secondary spurs formed on the primary long shoots of the Oil treatment did not differ significantly from the number of secondary spurs on the primary long shoots of the HC and TDZ treatments. The primary short shoots of the KNO_3 treatment had the lowest average number of secondary spurs formed (4.5), but did not differ significantly from the number of secondary spurs on the primary short shoots of the untreated control, Oil, KNO_3 + Oil, and the KNO_3 treatments.

Results from the 2020/2021 season:

Riebeek West. Reproductive bud break as a percentage of total dormant buds in the Riebeek West orchard did not differ significantly in the onset, maximum percentage and duration of reproductive bud break between the different RBAs and the untreated control (Fig. 6a; Table 14).

The vegetative bud break as a percentage of the total number of dormant buds (Fig. 6b) differed significantly in the onset (p<0.0001), percentage vegetative bud break at maximum reproductive bud break (p<0.0001), maximum percentage bud break (p=0.019) and the vegetative bud break period (p<0.0001) between the RBAs and the untreated control (Table 14). The HC + Oil, Oil, TDZ and KNO_3 + Oil treatments showed a significantly faster onset, compared to the rest of the treatments, while the untreated control had a significantly longer onset compared to all the other treatments. For the percentage vegetative bud break at maximum reproductive bud break, HC + Oil, Oil and KNO_3 + Oil had the highest percentage, but did not differ significantly from TDZ. The HC treatment did not differ significantly from the TDZ treatment or the untreated control, which, in turn, did not differ significantly from KNO_3, having the lowest percentage vegetative bud break at maximum reproductive bud break. The KNO_3 + Oil treatment again had the highest maximum percentage vegetative bud break but did not differ significantly from Oil and KNO_3. The KNO_3 + Oil treatment was the only treatment that differed significantly from the control and the control also having the lowest maximum percentage vegetative bud break. The total bud break period of the control was the shortest but did not differ significantly from that of KNO_3. The Oil treatment had the longest bud break period but did not differ significantly from any of the other treatments, except KNO_3 and the control.

Total bud break as a percentage of the total number of dormant buds (Fig. 6c) differed significantly in the onset (p=0.0007) and the maximum percentage total bud break (p=0.0311) (Table 14). The HC + Oil treatment had the shortest onset, which did not differ significantly from KNO_3 + Oil. The control had the longest onset, but did not differ significantly from HC, TDZ and KNO_3. None of the other treatments differed significantly from each other. The maximum percentage total bud break was greater than 86% for all the treatments, as well as the untreated control, in the Riebeek West orchard. The KNO_3 + Oil resulted in the highest maximum percentage total bud break, differing significantly only from KNO_3 and the control, which had the lowest maximum percentage total bud break. The total bud break period did not differ significantly between the different RBAs and the untreated control.

The fruit set ranged from 13.1 to 17.2% without differences for the different RBAs and the control and did not significantly interact with shoot type (Table 15). However, there was a significant difference in the percentage fruit set per shoot type (p<0.0001). Spurs had the highest average fruit set of 21.3%, followed by short shoots and lastly, long shoots, with the latter having a significantly lower average percentage fruit set (10.5).

Worcester. The reproductive bud break as a percentage of the total number of dormant buds for the Worcester orchard did not differ in the onset, maximum percentage, and period of bud break between the different RBAs and the untreated control (Fig. 7a; Table 16). The vegetative bud break as a percentage of the total dormant buds differed in the onset (p<0.0001) and the bud break period (p=0.012) between the various RBAs and the untreated control (Table 16). The HC + Oil and TDZ treatments had the quickest onset but did not differ significantly from Oil and HC, while the KNO_3 treatment had the longest onset but did not differ significantly from KNO_3 + Oil and the control. In vegetative bud break period, KNO_3 and the control had the shortest periods, but did not differ significantly from Oil, HC and KNO_3 + Oil. The TDZ treatment had the longest vegetative bud break period, but did not differ significantly from HC + Oil, HC and KNO_3 + Oil (Fig. 7b). No significant differences were found in the percentage vegetative bud break at maximum reproductive bud break, as well as the maximum percentage vegetative bud break. Total bud break as a percentage of the total number of dormant buds (Fig. 7c), did not differ in the onset, maximum percentage and bud break period among the different RBAs and the untreated control (Table 16).

Fruit set results ranged from 23.2 to 32.6% but did not significantly interact with the shoot type (Table 17). Likewise, no significant difference was found between the different RBAs and the untreated control. There was, however, a significant difference in the average fruit set (p<0.0001) per shoot type. The spurs set significantly more fruit (40.6%) compared to the short shoots (15.9%).

Discussion

Rest breaking treatments did not increase the maximum percentage reproductive bud break, nor did it influence the onset and duration thereof. This is in contrast with the chemical rest breaking response found by Son and Küden (2005) on apricot and plum. El-Sabagh (2014)

also found an increase in reproductive bud break in almond trees treated with gibberellic acid (GA_3) alone, and in combination with both 6-benzyl adenine (6-BA) and KNO_3. A plausible explanation could be that the cultivar used in their trial, Abied safaks, has a higher chill requirement compared to Independence, and/or less chill accumulation took place compared to our trials, but this was not indicated in the publication. Furthermore, GA_3 and 6-BA treatments are commonly used for growth stimulation in deciduous fruit trees (Elfving and Visser, 2006), while not necessarily used as chemical RBAs *per se*. It might be possible that the 'Abied safaks' almond trees accumulated sufficient chilling to overcome endodormancy prior to treatment application. Therefore, these treatments most likely uplifted the state of paradormancy by interrupting apical dominance, causing lateral bud break and development. When taking into consideration the chill requirement results for Independence reported in Paper 1, it is evident that this cultivar has a low level of endodormancy. Therefore, rest breaking treatments cannot enhance or advance the bud break pattern when the chill requirement has been satisfied, especially in reproductive buds, which have a lower chill requirement than vegetative buds, as speculated by Saure (1985).

The rest breaking treatments did not influence the average percentage fruit set in either seasons at both trial sites, which is congruent with fruit set results found by Sagredo (2008) in apple. El-Sabagh (2014), however, found an increase in percentage fruit set during two seasons in almond trees treated with GA_3 in combination with both 6-BA and KNO_3 at 4%, compared to the control. Our data did, however, show a higher average fruit set percentage on spurs compared to the other bearing positions, as supported by Tombesi et al. (2016) that almonds predominantly bear on spurs. Furthermore, no significant interaction was found between rest breaking treatments and primary shoot types in the average fruit set percentage. The inability to influence reproductive bud break and percentage fruit set compared to the control, explains why no significant differences were found in any of the yield parameters, nor in the yield efficiency. Furthermore, rest breaking treatments did not have a horticulturally significant effect on any of the post-harvest quality parameters. This is congruent with results on apples (North, 1991; Sagredo, 2008), peaches and nectarines (Leonel et al., 2014; North et al., 1988) treated with chemical RBAs.

The rest breaking treatments shortened the onset of vegetative bud break with treatments containing oil generally, showing the best results. The HC + Oil treatment advanced the onset most and by more than ten days in most cases, compare to the untreated control. At

the Riebeek West trial site, rest breaking treatments enhanced the number of vegetative bud break when reproductive bud break was at its maximum, with treatments containing oil, again, showing the best results. A faster onset and higher percentage vegetative bud break at the time when reproductive bud break was at its maximum, indicates that the rest breaking treatments accelerated the vegetative bud break while reproductive buds were breaking. Treatments that advanced the onset showed an extended vegetative bud break period, indicating that the date of final vegetative bud break was not necessarily advanced. Early development of reproductive and vegetative growth is dependent on reserves in the tree (Hansen, 1971). Quinlan and Preston (1968) reported that the competition between meristems is at its greatest during and immediately after flowering, while reproductive buds are reported as weak sinks from the time of flowering until significant fruit growth starts (Wardlaw, 1968). Vegetative bud break that takes place simultaneous with reproductive bud break could therefore increase competition for reserves as these newly formed leaves themselves are sinks and compete with the reproductive development and only become net exporters of photosynthetic assimilates once a third to a half of the final leaf area is attained (Wardlaw1968). This competition could possibly impair flower development, lowering fruit set and ultimately compromising yield. However, once early development is completed, the greater part of fruit and shoot growth is dependent on the photosynthates produced during the current season (Hansen, 1971). Marchi et al. (2005) reported that peach leaves start to export photosynthetic assimilates seven to ten days after vegetative bud break. Therefore, if vegetative development and subsequent growth is advanced, earlier photosynthesis could lead to more assimilates available for both vegetative and reproductive growth. As the results indicate that rest breaking treatments did not influence reproductive bud break, fruit set percentage or the yield efficiency, one could expect more than a week's advantage in photosynthate production for trees treated with HC + Oil, compared to the untreated control, without compromising the reproductive bud break, fruit set and yield.

The maximum percentage vegetative bud break was only increased at the Riebeek West orchard, during the 2020 spring. The KNO_3 + Oil treatment was the only treatment that had a significantly higher maximum percentage vegetative bud break, compared to the untreated control, increasing bud break by 13.5%. Oil causes oxidative stress in the buds (Erez et al., 1980), which in turn causes the enzymatic reduction of NO_3^- to NO (Yamasaki et al., 1999), leading to dormancy breaking. NO is a reactive nitrogen species that competes for oxygen by inhibiting cytochrome oxidase (Brown and Borutaite, 2002), while increasing the content of reactive oxygen species by inhibiting catalase activity (Brown, 1995). These effects are similar

to that of adequate winter chill (Nir et al., 1986). One could speculate that the synergistic effect of oil enforcing anaerobic condition and the nitrogenous compounds from KNO_3 causing dormancy breaking and stimulating growth, was the reason for enhanced vegetative bud break, compared to treatments directing only rest break, as well as the untreated control. External competition for water and nutrients could also affect the vegetative bud break as was possibly the case at the Worcester orchard in 2020, thus leading to a substantially lower maximum percentage vegetative bud break compared to the previous season, while the reproductive bud break remained fairly similar. Stress associated with water shortages during March and April, 2020, could have decreased the number of vegetative buds that were viable during the subsequent spring (Doll, 2017), therefore, showing no reaction to rest breaking treatments. The orchard in the Worcester region also had challenges managing weed growth (depicted in Appendix D), which could increase the competition with trees minimising the availability of nutrients and water in the soil. This could have exacerbated the stress experienced due to water shortage. For the Riebeek West orchard, no noticeable decrease in maximum vegetative bud break was seen between seasons, as is the case for Worcester, as standard irrigation practices and better weed management was applied. Data for the 2021 harvest is not included in this study, therefore, the effect of rest breaking treatments during the second season was only determined with regards to bud break and fruit set.

The systematic replacement of the bearing surface in almond is facilitated by new vegetative growth (Kester et al., 1996). Therefore, an increase in vegetative bud break could lead to a higher yield via increased number of bearing positions. Although the overall vegetative growth was not affected by rest breaking treatments in the Riebeek West region, treatments containing oil, HC + Oil in particular, had a significantly higher growth index for secondary spurs. This is of great importance, as ‘Independence’ almond trees typically tend to have a higher set percentage on spurs. An increase in the spur growth index can therefore possibly increase the bearing surface while utilising less resources for vegetative growth, compared to the production of longer shoot types, thereby increasing the productivity in the bearing surface. There was, however, an interaction between the treatments and the primary shoot types, in the number of spurs formed. The increase in the average number of secondary spurs formed on primary long shoots after each treatment, is not necessarily similar to the increase on primary short shoots, after the same treatment application. Therefore, the different rest breaking treatments did not necessarily affect the different primary shoot types to the same extent, compared to the untreated control. However, it would be erroneous not to note that

treatments containing oil induced the highest average number of secondary spurs irrespective of primary shoot type, thus explaining the higher secondary spur growth index for these treatments.

The Worcester orchard showed similar results for the secondary spur growth index, with treatments containing oil, generally, increasing the growth index compared to other treatments and the untreated control. The interaction between the treatments and primary shoot type, however, indicated that the rest breaking treatments did not affect different primary shoot types in the same manner, nor to the same extent, in the average number of secondary spurs produced. Furthermore, treatments such as HC + Oil, HC and TDZ decreased the average number and growth index of secondary short shoots, therefore, leading to a decrease in the overall secondary growth index. Stress related conditions such as water shortage and possible competition for available nutrients as seen in the Worcester orchard, could lead to some rest breaking treatment increasing the secondary spur growth index, but at the cost of secondary short shoots, as shown in our results. In a young orchard where trees have not yet filled their allotted space, this increase in spur production could limit the growth of the trees which will prevent the orchard from reaching its full bearing potential over time. To address this, spur pruning can be implemented to renew and invigorate bearing wood. Spurs in almond trees remain viable for typically five years (Krueger et al., 1996), emphasizing the need for pruning to remove less productive wood and stimulate renewed growth.

Conclusion

According to our results, the rest breaking treatments had no effect on the reproductive bud break patterns of ‘Independence’ almond trees, nor did it affect the fruit set, yield efficiency or any of the post-harvest quality parameters. Our results did, however, show a higher percentage fruit set on spurs, compared to other bearing positions. Therefore, rest breaking treatments cannot be used to advance flowering in a cross-pollinizer if this was to bloom later than the main cultivar in low-chill self-incompatible almond cultivars.

Furthermore, rest breaking treatments that contained oil, HC + Oil in particular, shortened the onset of vegetative bud break, while enhancing the number of vegetative bud break when reproductive bud break is at its maximum. These treatments generally caused a greater overlap between reproductive and vegetative bud break, which could lead to higher

competition among vegetative and reproductive development, ultimately compromising fruit set and yield. However, the results in this study showed that this was not the case for fruit set in both seasons, as well as yield during the first season. On the other hand, the sooner vegetative bud break can commence, the sooner the sink-source transition for newly formed leaves can take place, advancing the photosynthetic capacity of a tree. This could increase the amount of available photosynthetic assimilates during fruit set and growth, as well as the reserves available for bud break and early development in the subsequent spring.

Rest breaking treatments generally did not influence overall vegetative growth, but did, however, increase spur formation. Treatments containing oil, especially HC + Oil, enhanced spur formation and, therefore, increased the potential bearing positions for the subsequent season. However, under stress related conditions, spur formation took place at the expense of shoot growth, therefore, decreasing the overall secondary vegetative growth. This could be problematic in young trees that have not yet filled their allotted space in the orchard. If rest breaking treatments are considered from the first year after planting, it would be advised to plant trees at a higher density, ensuring the allotted space is filled.

It should be noted that bud break and fruit set data from this study is based on the results from two seasons, while vegetative growth indexes, yield and post-harvest quality parameters is based on results from a single season. According to the results from this study, the 0.5% HC + 2% Oil treatment proved to be the most efficient chemical RBA in improving vegetative bud break and increasing the potential bearing positions in ‘Independence’ almond trees. However, an increase in spur formation under stress related conditions could compromise overall vegetative growth. This can be addressed by pruning to ensure renewal growth, as well as eliminating less productive wood that might compromise light management in trees.

Literature Cited

Alonso, J.M. 2017. Environmental requirements, p. 254-278. In: R. Socias i Company and T.M. Gradziel (eds.). Almond: botany, production and uses. CABI, Boston, MA.

Australian Almonds. 2020. Almond insights 2019-20. Australian Board of Almonds, Loxton.

Brown, C. 1995. Reversible binding and inhibition of catalase by nitric oxide. Eur. J. Biochem. 232:188-191.

Brown, C. and V. Borutite. 2002. Nitric oxide inhibition of mitochondrial respiration and its role in cell death. Free Radic. Biol. Med. 33(11):1440-1450.

California Department of Food and Agriculture. 2019. 2018 California almond acreage report. CDFA, Sacramento, CA, USA.

Cape Almonds. 2017. Prospectus: Independence cultivar. ZZ2, Mooketsi, South Africa.

Costa, C., P.J.C Stassen and J. Mudzunga. 2004. Chemical rest breaking agents for the South African pome and stone fruit industry. Acta Hort. 636:295-302.

Doll, D. 2017. Almond water requirements, p. 279-290. In: R. Socias i Company and T.M. Gradziel (eds.). Almond: botany, production and uses. CABI, Boston, MA.

Elfving, D.C. and D.B. Visser. 2006. The use of bioregulators in the production of deciduous fruit trees. Acta Hort. 727:57-66.

El-Sabagh, A.S. 2014. Influence of potassium nitrate, gibberellin and benzyl adenine on bud break, fruit set and branch induction of almond Trees. Acta Hort. 1028:259-365.

Erez, A. 1987. Chemical control of bud break. HortScience 22:1240-1243.

Erez, A. 2000. Bud dormancy; phenomenon, problems and solutions in the tropics and subtropics, p. 17-48. In: A. Erez (ed.). Temperate fruit crops in warm climates. Kluwer Academic Publishers, Institute of Horticulture, Bet Dagan, Israel.

Erez, A., G.A. Couvillon, and S.J. Kays. 1980. The effect of oxygen concentration on the release of peach leaf buds from rest. HortScience 15:39-41.

Faust, M., M.M. Millard, and G.W. Stutte. 1991. Bound versus free water in dormant apple buds – a theory for endodormancy. HortScience 26:887-890.

Glozer, K., and Coates, W.W. (2006). Comparison of CPPU, Dormex, CAN17 and other treatments in dormant season applications on sweet cherry in 2006. <https://ucanr.edu/repositoryfiles/425-56-62417.pdf.>

Godini, A. 2002. Almond fruitfulness and role of self-fertility. Acta Hort. 591:191-203.

Gradziel, T.M., R. Curtis and R. Socias i Company. 2017. Production and growing regions, p. 70-86. In: R. Socias i Company and T.M. Gradziel (eds.). Almond: botany, production and uses. CABI, Boston, MA.

Hansen, P. 1971. ^{14}C-Studies on Apple Trees. VII. The early seasonal growth in leaves, flowers and shoots as dependent upon current photosynthates and existing reserves. Physiol. Plant. 25:469-473.

Kester, D.E., G.C. Martin, and J.M. Labavitch. 1996. Growth and development, p. 90-97. In: W.C. Micke (ed.). Almond production manual. Univ. Calif., Richmond, CA.

Krueger, W., J.H. Connell, and M.W. Freeman. 1996. Pruning bearing trees, p. 125-131. In: W.C. Micke (ed.). Almond Production Manual. Univ. Calif., Richmond, CA.

Leonel, S., Leonel, M., and M.A. Tecchio. 2014. Fruit quality in the peach and nectarine with application of hydrogenated cyanamide and mineral oil. Rev. Ciênc. Agron. 45(3):581-587.

Linsley-Noakes, G.C., G. Matthee, and P. Allan. 1994. Modification of rest completion prediction models for improved accuracy in South African stone fruit orchards. J. S. Afr. Soc. Hort. Sci. 4:13-15.

Lloyed, J. and D.J. Firth. 1993. Effect of hydrogen cyanamide and Promalin® on flowering, fruit set and harvest time of 'Flordaprince' peach (*Prunus persica* L. Batsch) in subtropical Australia. J. Hort. Sci. 68:177-183.

Luedeling, E. and P.H. Brown. 2011. A global analysis of the comparability of winter chill models for fruit and nut trees. Int. J. Biometeorol. 55:411-421.

Marchi, S., L. Sebastiani, R. Gucci, and R. Tognetti. 2005. Sink-source transition in peach leaves during shoot development. J. Amer. Soc. Hort. Sci. 130(6):928-935.

Niederholzer, F.J.A. and K. Glozer. 2019. Bloom timing and fruit set of 'Improved French' prune following dormant application of horticultural oil or nitrogen fertilizers as res-breaking agents. Acta Hort. 1260:117-124.

Nir, G., J. Schulman, L. Fanberstein, and G. Lvee. 1986. Changes in the activity of catalase in relation to the dormancy of grapevine. Plant Physiol. 81(4):1140-1142.

North, M.S. 1991. Alternative rest breaking agents to DNOC/oil for apples. S. Afr. J. Plant Soil 9(1):39-40.

North, M.S., J.D. Stadler, and H. de Villiers. 1988. Effect of cyanamide and girdling on yield, fruit size and quality of 'De Wet' peach. S. Afr. J. Plant Soil 5(4):224-226.

Ortega, E., J. Egea, and F. Dicenta. 2004. Effective pollination period in almond cultivars. HortScience 39:19–22.

Prudencio, A.S., P. Martínez-Gómez, and F. Dicenta. 2018. Evaluation of breaking dormancy, flowering and productivity of extra-late and ultra-late flowering almond cultivars during cold and warm seasons in South-East of Spain. Sci. Hortic. 235:39-46.

Quinlan, J.D. and A.P. Preston. 1968. Effects of thinning blossom and fruitlets on growth and cropping of Sunset apple. J. hort. Sci. 43:737-381.

Sagredo, K.X. 2008. Effect of rest-breaking and fruit thinning treatments on reproductive development in apple. Stellenbosch Univ., South Africa, PhD Diss. 62-85.

Sarvisé, R. and R. Socias i Company. 2004. Variability and heritability of bud density and branch habit in almond. Acta Hort. 663:401-404.

Saure, M.C. 1985. Dormancy release in deciduous fruit trees. Hort. Rev. 7:239-300.

Sheard, A.G., S.D. Johnson, and N.C. Cook. 2009. Effect of timing and concentration of rest breaking agents on budburst in 'Bing' sweet cherry under conditions of inadequate winter chilling in South Africa. S. Afr. J. Plant Soil. 26(2):73-79.

Shulman, Y., G. Nir, and S. Lavee. 1986. Oxidative processes in bud dormancy and the use of hydrogen cyanamide in breaking dormancy. Acta Hort. 179:141-148.

Socias i Company, R. J.M. Ansón, and M.T. Espiau. 2017. Taxonomy, botany and physiology, p. 1-42. In: R. Socias i Company and T.M. Gradziel (eds.). Almonds: botany, production and uses. CABI, Boston, MA.

Son, L. and A.B. Küden. 2005. Dormex and Promalin affects fruit set and earliness of apricot (*Prunus armeniaca*) and plum (*Prunus domestica*) cultivars. New Zeal. J. Crop. Hort. 33:59-64.

Strydom, D.K., G.E. Honeyborne, and G.J. Eksteen. 1971. Delayed foliation of pome- and stonefruit. Deciduous Fruit Grower 21:126.129.

Szalay, L. 2006. Comparison of flower bud development in almond, apricot and peach genotypes. Int. J. Hort. Sci. 12(2):93-98.

Tombesi, S., B.D. Lampinen, S. Metcalf, and T.M. DeJong. 2016. Yield in almond is related more to the abundance of flowers than the relative number of flowers that set fruit. Calif. Agric. 71(2):68-74.

Wardlaw, I.F. 1968. The control and pattern of movement of carbohydrates in plants. Biol. Rev. 34:79-105.

Yamasaki, H., Y. Sakihama, and S. Takahashi. 1999. An alternative pathway for nitric oxide production in plants: new features of an old enzyme. Trends Plant Sci. 4:128-129.

Fig. 1.1. Scoring chart to determine pellicle colour of ‘Independence’ almond kernels. Numbers indicate increasing shades of brown with 1 respresenting light brown and 5 representing very dark brown.

Fig. 1.2. Scoring chart to determine roughness of ‘Independence’ almond kernels. Numbers indicate increasing roughness with 1 representing smooth and 3 representing wrinkeled.

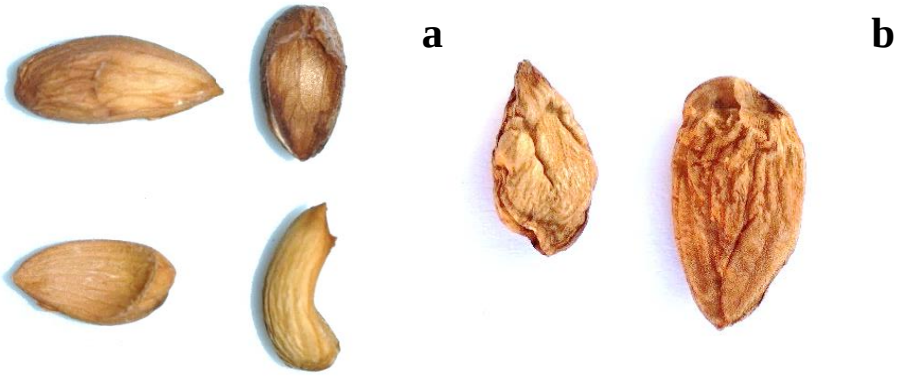


Fig. 1.3. a) Double and b) shrivelled kernel indicating the defects in ‘Independence’ kernels.

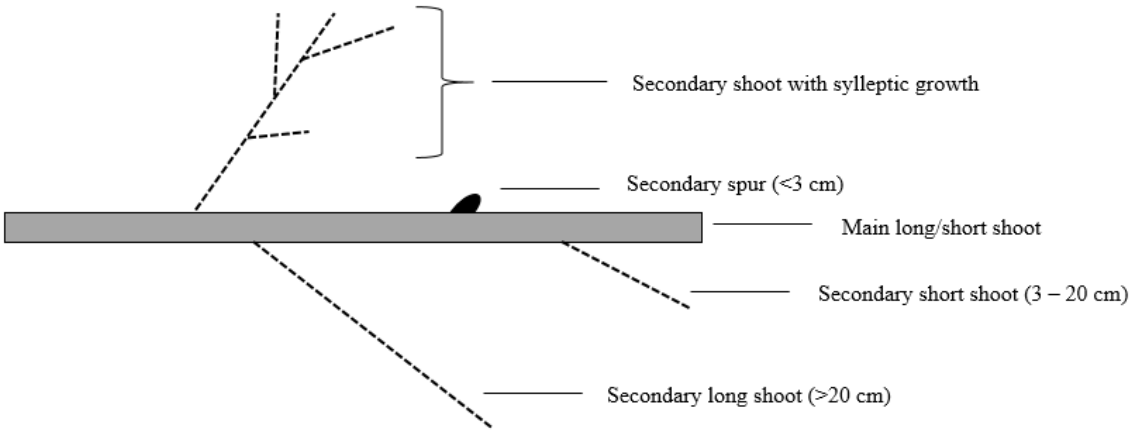


Fig. 2. Visial representation of the primary shoot and all four secondary shoot types, as used to describe the growth index of 'Independence' almond trees.

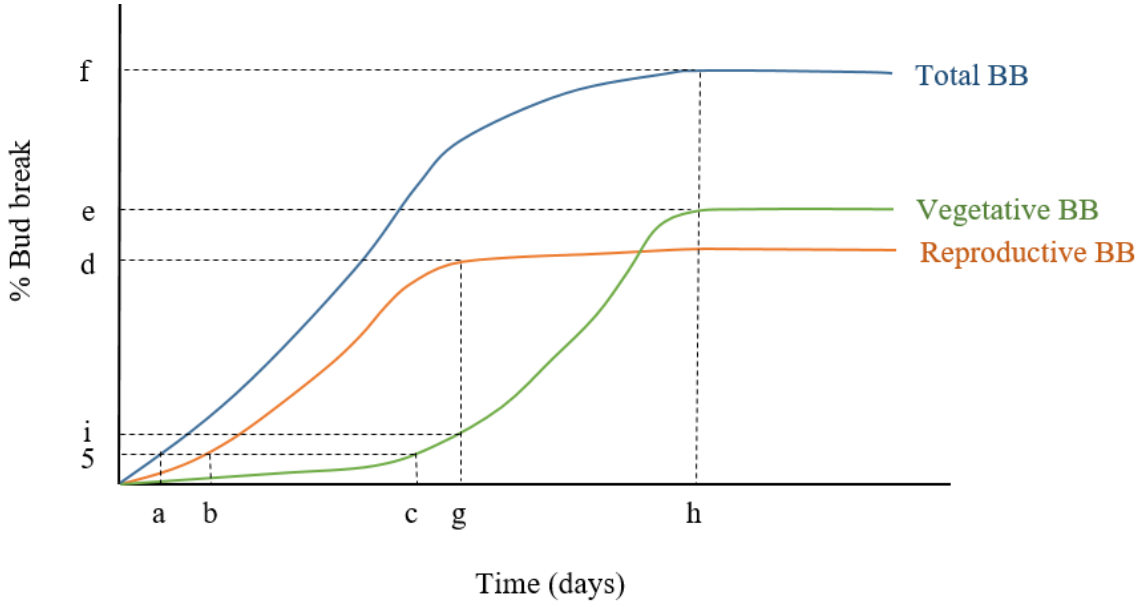


Fig. 3. Visual representation of the total, reproductive and vegetative bud break (BB) patterns as a percentage of the total number of dormant buds. Parameters include the number of days until 5% BB - onset (a – c), the maximum percentage BB (d – f), the period of BB (h minus a; g minus b; h minus c) and the percentage vegetative BB when reproductive BB is at its maximum (i). Total BB is the sum of reproductive and vegetative BB.

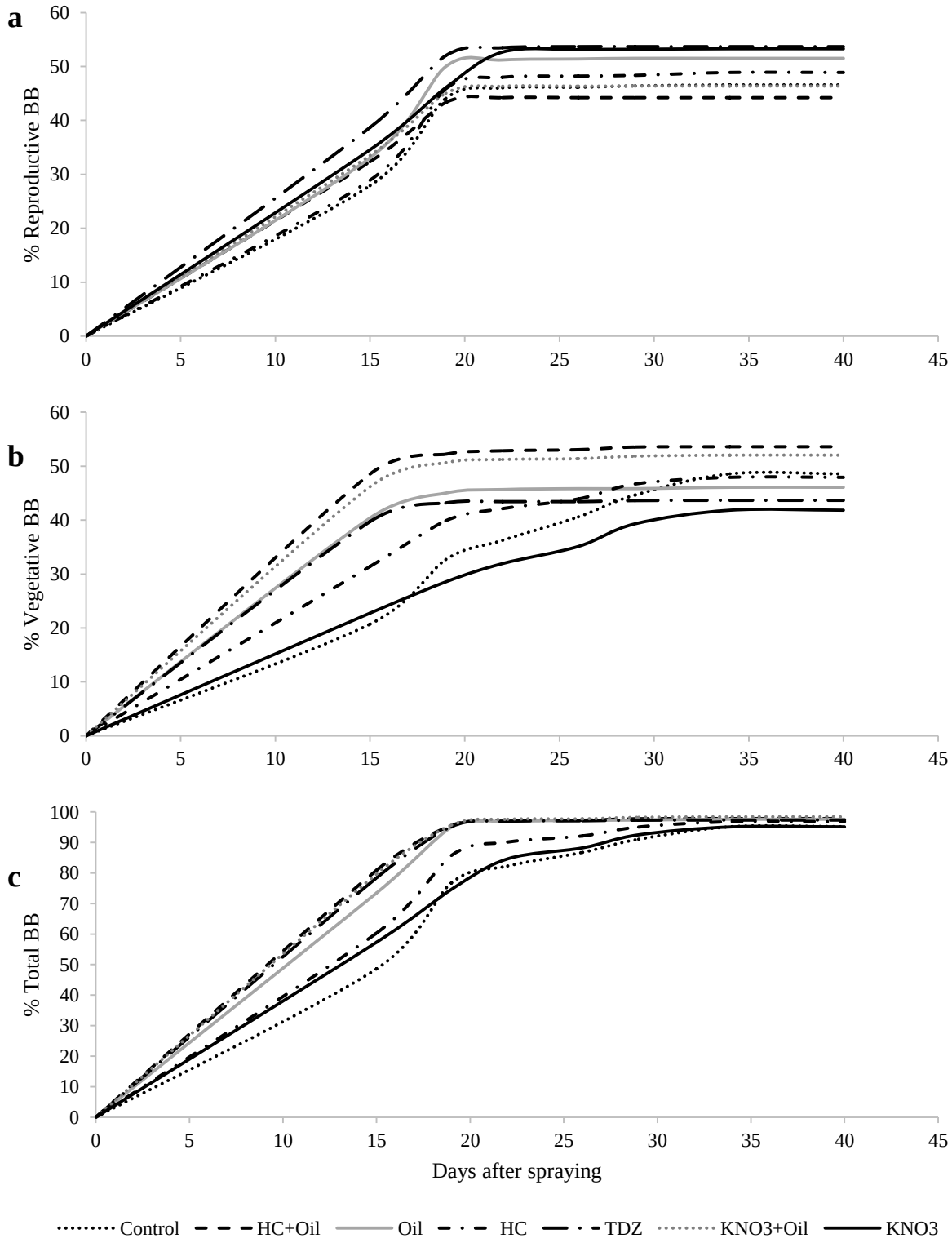


Fig. 4. Percentage bud break (BB) curves for the a) reproductive, b) vegetative and c) total number of buds (reproductive + vegetative) for the different treatments and the control at the Groenrivier 'Independence' trial site, (Riebeek West) during the 2019 spring. Treatment concentrations were as follow: 0.5% HC + 2% Oil; 2% Oil; 0.5% HC; 2.5% TDZ; 50 g/L KNO_3 + 2% Oil; 50 g/L KNO_3. HC = hydrogen cyanamide; TDZ = thidiazuron

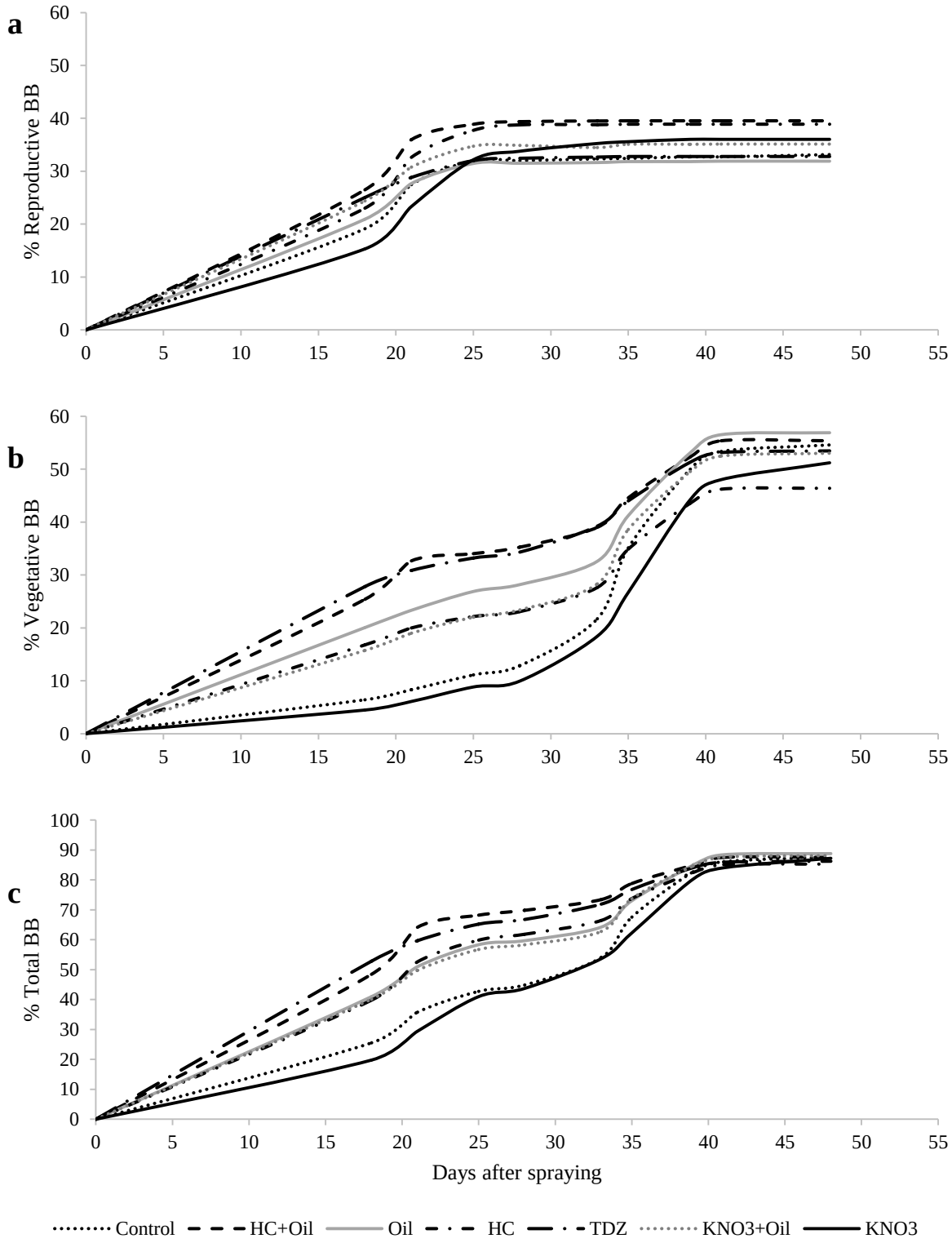


Fig. 5. Percentage bud break (BB) curves for the a) reproductive, b) vegetative and c) total number of buds (reproductive + vegetative) for the different treatments and the control at Hex Poort 'Independence' trial site, (Worcester) during the 2019 spring. Treatment concentrations were as follow: 0.5% HC + 2% Oil; 2% Oil; 0.5% HC; 2.5% TDZ; 50 g/L KNO_3 + 2% Oil; 50 g/L KNO_3. HC = hydrogen cyanamide; TDZ = thidiazuron

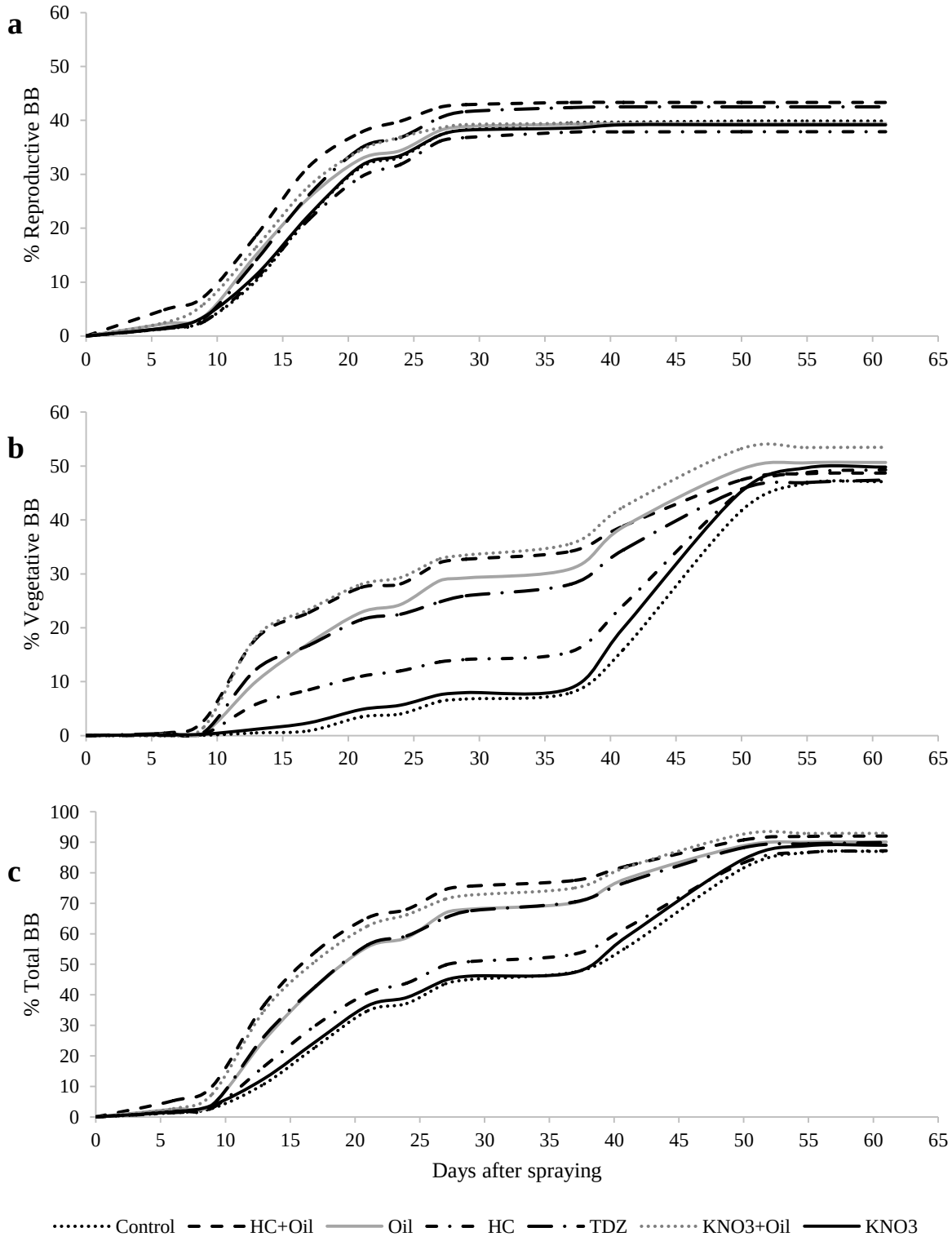


Fig. 6. Percentage bud break (BB) curves for the a) reproductive, b) vegetative and c) total number of buds (reproductive + vegetative) for the different treatments and the control at Groenrivier 'Independence' trial site, (Riebeek West) during the 2020 spring. Treatment concentrations were as follow: 0.5% HC + 2% Oil; 2% Oil; 0.5% HC; 2.5% TDZ; 50 g/L KNO_3 + 2% Oil; 50 g/L KNO_3. HC = hydrogen cyanamide; TDZ = thidiazuron

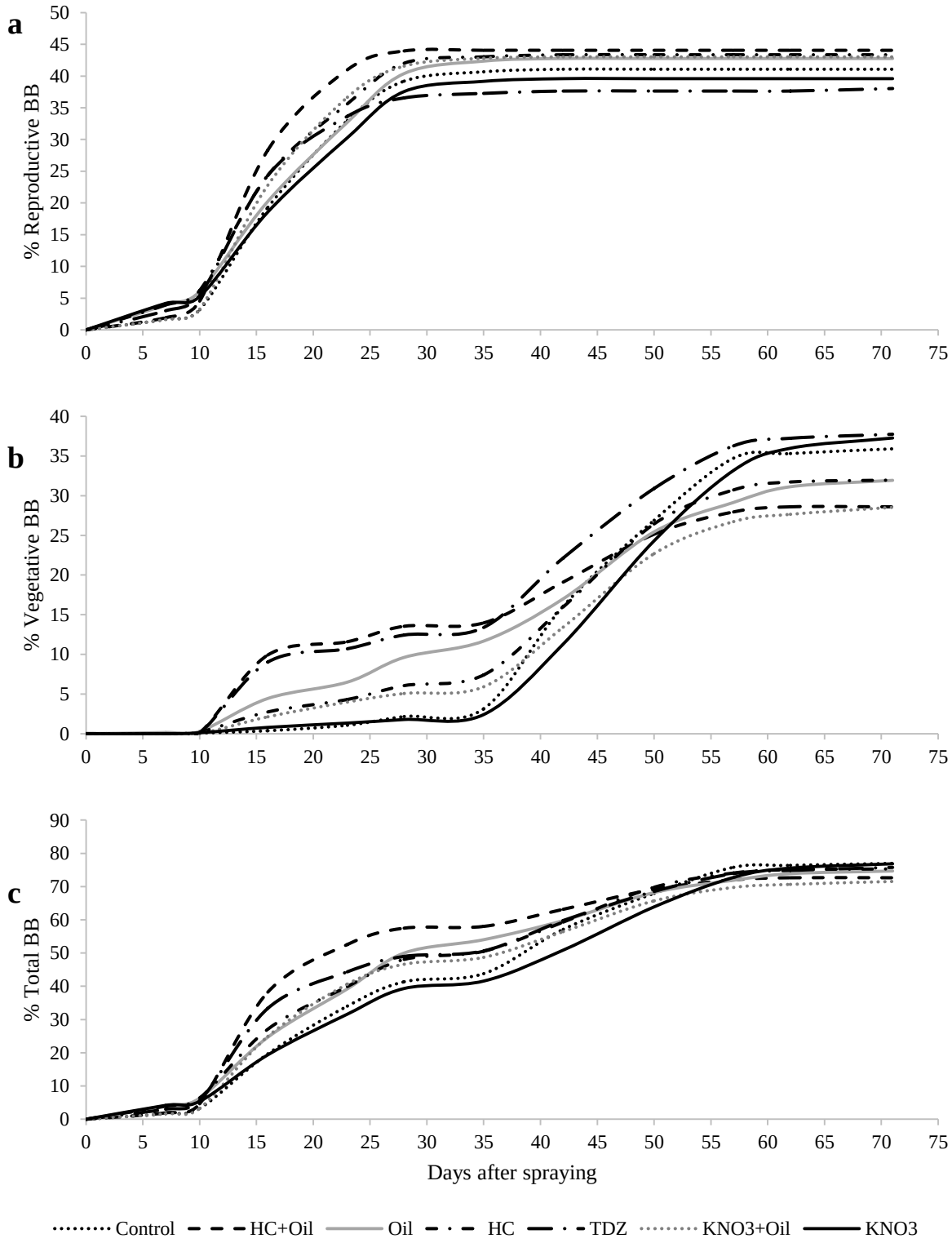


Fig. 7. Percentage bud break (BB) curves for the a) reproductive, b) vegetative and c) total number of buds (reproductive + vegetative) for the different treatments and the control at Hex Poort 'Independence' trial site, (Worcester) during the 2020 spring. Treatment concentrations were as follow: 0.5% HC + 2% Oil; 2% Oil; 0.5% HC; 2.5% TDZ; 50 g/L KNO_3 + 2% Oil; 50 g/L KNO_3. HC = hydrogen cyanamide; TDZ = thidiazuron

Table 1. Treatment specifications for rest breaking agents applied over two consecutive seasons on both the Groenrivier (Riebeek West) and Hex Poort (Worcester) 'Independence' almond trial sites during August 2019 and 2020.

Treatment abbreviation	Product name	Active ingredient	$g \cdot L^{-1}$ active ingredient	Percentage product	Manufacturing company	Country
Untreated control	N/A	None	N/A	N/A	N/A	N/A
HC	Dormex®	Hydrogen cyanamide	2.6	0.5%	AlzChem Group AG	Trosberg, Germany
Oil	Opron®	Mineral oil	17	2%	H&R	Bluff, South Africa
HC + Oil	Dormex® + Opron®	Hydrogen cyanamide + Mineral oil	2.6 + 17	0.5% and 2%	AlzChem Group AG; H&R	Trosberg, Germany; Bluff, South Africa
TDZ	Lift®	Thidiazuron	0.075	2.5%	Almond Agro Chemicals	Kempton Park, South Africa
KNO_3	KNO_3	Potassium nitrate	50	N/A	Haifa Chemicals	Brackenfell, South Africa
KNO_3 + Oil	KNO_3 + Opron®	Potassium nitrate + Mineral oil	50 + 17	N/A and 2%	Haifa Chemicals; H&R	Brackenfell, South Africa; Bluff, South Africa

Table 2. Effect of different rest breaking agents on the reproductive bud break (BB), vegetative BB and total BB as a percentage of the total number of dormant buds, as well as fruit set of the 'Independence' almond orchard at Groenrivier (Riebeek West) during the 2019 spring. Treatment concentrations were as follow: 0.5% HC + 2% Oil; 2% Oil; 0.5% HC; 2.5% TDZ; 50 g/L KNO_3 + 2% Oil; 50 g/L KNO_3. HC = hydrogen cyanamide; TDZ = thidiazuron; BB = bud break; BBveg@maxBBrep = Vegetative BB at maximum reproductive BB. Means, within each column, with the same letter are not significantly different. ns = no significant difference.

Treatment	Reproductive BB			Vegetative BB				Total BB			Fruit set (%)
	Onset *(days)	Maximum (%)	BB period (days)	Onset *(days)	BB_{veg} @ max BB_{rep} (%)	Maximum (%)	BB period (days)	Onset *(days)	Maximum (%)	BB period (days)	
Control	3.14 ns	46.59 ns	21.26 ns	4.04 a	40.22 bc	48.58 abc	28.66 a	1.72 ns	95.17 b	30.98 a	38.79 ns
HC + Oil	2.97	44.22	17.23	1.59 c	52.72 a	53.60 a	21.11 c	0.95	97.79 a	22.15 b	39.94
Oil	2.79	51.51	19.61	1.94 bc	45.58 ab	46.08 bc	22.76 bc	1.54	97.59 a	23.86 b	41.02
HC	2.92	48.89	21.78	2.59 b	45.59 ab	47.93 abc	27.51 a	1.31	96.82 ab	28.79 a	40.43
TDZ	2.22	53.68	19.68	1.93 bc	43.33 b	43.66 c	21.47 c	0.98	97.34 a	24.12 b	42.40
KNO_3 + Oil	2.64	49.98	18.86	1.74 c	50.98 a	52.04 ab	21.26 c	1.01	98.42 a	23.19 b	36.81
KNO_3	3.47	53.28	21.63	3.55 a	35.36 c	41.83 c	27.45 ab	1.39	95.12 b	29.61 a	36.69
Significance level	*0.8609*	*0.2186*	*0.0958*	***<0.0001***	***0.0001***	***0.0243***	***0.0017***	*0.0764*	***0.0074***	***0.0003***	*0.4025*
LSD %	-	-	-	*0.66*	*7.18*	*7.36*	*4.73*	-	*2.01*	*4.46*	-

* days to 5% BB from application of rest breaking treatment

Table 3. Quality differences in 'Independence' almonds of trees treated with different rest breaking agents at Groenrivier, Riebeek West, Western Cape (2019/2020). Treatment concentrations were as follow: 0.5% HC + 2% Oil; 2% Oil; 0.5% HC; 2.5% TDZ; 50 g/L KNO_3 + 2% Oil; 50 g/L KNO_3. HC = hydrogen cyanamide; TDZ = thidiazuron. Means, within each column, with the same letter are not significantly different. ns = no significant difference.

Treatment	Nut weight per tree (kg)			Percentage Kernel	Yield Efficiency ($g.cm^{-2}$)	Individual Kernel	
	Dry	In-Shell	Kernel			Weight (g)	Length (mm)
Control	9.5 ns	4.5 ns	2.4 ns	55.1 ns	27.23 ns	1.17 ns	23.62 ab
HC + Oil	9.4	4.4	2.5	57.7	29.33	1.19	23.91 a
Oil	9.4	4.5	2.5	57.1	28.89	1.17	23.60 ab
HC	6.4	2.9	1.6	58.6	23.58	1.21	23.98 a
TDZ	8.9	4.2	2.5	60.7	32.28	1.20	23.67 ab
KNO_3 + Oil	9.1	4.3	2.4	54.9	29.40	1.19	23.57 ab
KNO_3	8.3	3.7	2.1	58.7	25.31	1.18	23.26 b
Significance level	*0.2643*	*0.1066*	*0.0795*	*0.6095*	*0.3285*	*0.4823*	***0.0268***
LSD 5%	-	-	-	-	-	-	*0.41*

Table 4. Quality differences in 'Independence' almonds of trees treated with different rest breaking agents at Groenrivier, Riebeek West, Western Cape (2019/2020). Treatment concentrations were as follow: 0.5% HC + 2% Oil; 2% Oil; 0.5% HC; 2.5% TDZ; 50 g/L KNO_3 + 2% Oil; 50 g/L KNO_3. HC = hydrogen cyanamide; TDZ = thidiazuron. Means, within each column, with the same letter are not significantly different. ns = no significant difference.

Treatment	Individual Kernel				Percentage of Kernels	
	Width (mm)	Thickness (mm)	Colour	Roughness	Double	Shrivelled
Control	12.12 ns	8.32 bc	2.72 a	1.64 b	0.22 ns	0.46 ns
HC + Oil	12.36	8.25 c	2.50 c	1.62 b	0.27	0.54
Oil	12.26	8.36 b	2.38 d	1.64 b	0.14	0.85
HC	12.45	8.42 ab	2.55 bc	1.71 a	0.45	0.50
TDZ	12.25	8.49 a	2.54 bc	1.54 c	0.34	0.71
KNO_3 + Oil	13.38	8.35 b	2.53 bc	1.67 ab	0.54	0.52
KNO_3	12.15	8.41 ab	2.64 ab	1.71 a	0.45	0.66
Significance level	*0.3681*	***0.0003***	***<0.0001***	***<0.0001***	*0.4712*	*0.8064*
LSD 5%	-	*0.10*	*0.11*	*0.07*	-	-

Table 5. The average growth index in terms of all secondary growth, secondary shoots with sylleptic growth, secondary long shoot growth, secondary short shoot growth and secondary spur growth for 'Independence' almond trees in Riebeek West during the 2019/2020 season. Treatment concentrations were as follow: 0.5% HC + 2% Oil; 2% Oil; 0.5% HC; 2.5% TDZ; 50 g/L KNO_3 + 2% Oil; 50 g/L KNO_3. HC = hydrogen cyanamide; TDZ = thidiazuron.

Treatment	Growth index				
	All secondary growth	Secondary sylleptic shoot	Secondary long shoot	Secondary short shoot	Secondary spur
Control	1.01 ns	0.11 ns	0.23 ns	0.36 ns	0.26 c
HC + Oil	1.01	0.00	0.12	0.37	0.52 a
Oil	0.85	0.00	0.12	0.31	0.42 b
HC	0.96	0.14	0.14	0.38	0.29 c
TDZ	0.82	0.00	0.10	0.30	0.43 b
KNO_3 + Oil	0.99	0.04	0.14	0.37	0.44 b
KNO_3	0.81	0.01	0.15	0.42	0.24 c
Significance level	*0.3465*	*0.0600*	*0.5290*	*0.5276*	***<0.0001***
LSD 5%	-	-	-	-	*0.08*

Table 6. The interaction between the different rest breaking treatments and the primary shoot type on the average number of secondary shoots with sylleptic growth and spurs formed at the Groenrivier (Riebeek West) 'Independence' trial site during the 2019/2020 season. Treatment concentrations were as follow: 0.5% HC + 2% Oil; 2% Oil; 0.5% HC; 2.5% TDZ; 50 g/L KNO_3 + 2% Oil; 50 g/L KNO_3. HC = hydrogen cyanamide; TDZ = thidiazuron. Means, within each column, with the same letter are not significantly different.

Treatment combination		Average number of secondary growth	
		Sylleptic shoots	Spurs
Control	Long shoot	0.6 a	37.7 c
	Short shoot	0.0 b	8.7 f
HC +Oil	Long shoot	0.0 b	74.1 a
	Short shoot	0.0 b	20.9 d
Oil	Long shoot	0.0 b	61.9 b
	Short shoot	0.0 b	14.3 def
HC	Long shoot	0.6 a	42.5 c
	Short shoot	0.0 b	9.5 ef
TDZ	Long shoot	0.0 b	60.3 b
	Short shoot	0.0 b	15.6 def
KNO_3 + Oil	Long shoot	0.3 ab	60.2 b
	Short shoot	0.0 b	18.6 de
KNO_3	Long shoot	0.1 b	34.5 c
	Short shoot	0.0 b	9.2 ef
Significance level			
Treatment		***0.0470***	***<0.0001***
Primary shoot type		***0.0014***	***<0.0001***
*Treatment*primary shoot type*		***0.0470***	***0.0004***
LSD 5%			
Treatment		*0.26*	*6.82*
Primary shoot type		*0.14*	*3.64*
*Treatment*primary shoot type*		*0.37*	*9.79*

Table 7. The effect of different rest breaking treatments and primary shoot type on the average number of secondary long and short shoots formed at the Groenrivier (Riebeek West) 'Independence' trial site during the 2019/2020 season. Treatment concentrations were as follow: 0.5% HC + 2% Oil; 2% Oil; 0.5% HC; 2.5% TDZ; 50 g/L KNO_3 + 2% Oil; 50 g/L KNO_3. HC = hydrogen cyanamide; TDZ = thidiazuron. Means, within each column, with the same letter are not significantly different. ns = no significant difference.

Treatment	Average number of secondary growth	
	Long shoots	Short shoots
Rest breaking treatment		
Control	0.85 ns	6.85 ns
HC + Oil	0.45	7.20
Oil	0.45	6.20
HC	0.70	6.70
TDZ	0.45	5.85
KNO_3 + Oil	0.60	6.75
KNO_3	0.60	7.65
Primary shoot type		
Long shoot	0.97 a	10.31 a
Short shoot	0.20 b	3.17 b
Significance level		
Treatment	*0.6415*	*0.7827*
Primary shoot type	***<0.0001***	***<0.0001***
*Treatment*Primary shoot type*	*0.7233*	*0.5026*
LSD 5%		
Treatment	-	-
Primary shoot type	*0.27*	*1.22*
*Treatment*Primary shoot type*	-	-

Table 8. Effect of different rest breaking agents on the reproductive bud break (BB), vegetative BB and total BB as a percentage of the total number of dormant buds, as well as fruit set of the 'Independence' almond orchard at Hex Poort (Worcester) during the 2019 spring. Treatment concentrations were as follow: 0.5% HC + 2% Oil; 2% Oil; 0.5% HC; 2.5% TDZ; 50 g/L KNO_3 + 2% Oil; 50 g/L KNO_3. HC = hydrogen cyanamide; TDZ = thidiazuron; BB = bud break; BBveg@maxBBrep = Vegetative BB at maximum reproductive BB. Means, within each column, with the same letter are not significantly different. ns = no significant difference.

Treatment	Reproductive BB			Vegetative BB				Total BB			Fruit set (%)
	Onset *(days)	Maximum (%)	BB period (days)	Onset *(days)	BB_{veg} @ max BB_{rep} (%)	Maximum (%)	BB period (days)	Onset *(days)	Maximum (%)	BB period (days)	
Control	7.10 ns	33.15 ns	23.20 ns	17.82 a	24.34 ns	54.37 ns	28.18 bc	4.63 ns	87.75 ns	36.27 ns	30.38 ns
HC + Oil	4.71	39.54	24.89	5.11 bc	35.97	55.37	35.09 ab	3.51	87.55	36.69	34.14
Oil	5.81	31.90	23.29	7.12 bc	32.04	56.90	35.68 ab	2.62	88.81	40.19	34.15
HC	5.45	38.89	25.85	10.10 b	24.44	46.43	31.41 ab	4.26	85.29	37.24	34.73
TDZ	4.20	32.79	24.40	3.74 c	35.96	53.46	36.96 a	1.78	86.26	38.92	33.89
KNO_3 + Oil	4.13	35.14	26.77	7.44 bc	29.89	53.03	34.06 ab	2.55	88.17	38.95	35.71
KNO_3	7.35	32.83	24.15	19.37 a	18.28	51.22	23.73 c	5.22	87.25	37.88	31.38
Significance level	*0.3151*	*0.5056*	*0.8681*	***<0.0001***	*0.0799*	*0.4805*	***0.0113***	*0.0566*	*0.9553*	*0.1911*	*0.5834*
LSD %	-	-	-	*5.37*	-	-	*7.66*	-	-	-	-

* days to 5% BB from application of rest breaking treatment

Table 9. Quality differences in 'Independence' almonds of trees treated with different rest breaking agents at Hex Poort, Worcester, Western Cape (2019/2020). Treatment concentrations were as follow: 0.5% HC + 2% Oil; 2% Oil; 0.5% HC; 2.5% TDZ; 50 g/L KNO_3 + 2% Oil; 50 g/L KNO_3. HC = hydrogen cyanamide; TDZ = thidiazuron Means with the same letter are not significantly different. ns = no significant difference.

Treatment	Nut weight per tree (kg)			Percentage Kernel	Yield Efficiency ($g.cm^{-2}$) Dry	Individual Kernel	
	Dry	In-Shell	Kernel			Weight (g)	Length (mm)
Control	3.0 ns	1.4 ns	0.8 ns	58.38 ns	10.54 ns	0.95 ns	22.71 a
HC + Oil	3.3	1.7	1.0	57.79	19.98	0.90	22.23 bc
Oil	3.1	1.6	0.9	58.80	12.02	0.90	22.48 abc
HC	3.2	1.6	0.9	56.74	11.03	0.89	22.07 c
TDZ	3.3	1.7	0.9	55.57	10.53	0.91	22.27 abc
KNO_3 + Oil	3.6	1.8	1.1	57.78	13.67	0.89	22.12 c
KNO_3	3.3	1.6	0.9	58.58	11.71	0.94	22.66 ab
Significance level	*0.7616*	*0.5010*	*0.6248*	*0.8217*	*0.0873*	*0.1880*	***0.0500***
LSD 5%	-	-	-	-	-	-	*0.48*

Table 10. Quality differences in 'Independence' almonds of trees treated with different rest breaking agents at Hex Poort, Worcester, Western Cape (2019/2020). Treatment concentrations were as follow: 0.5% HC + 2% Oil; 2% Oil; 0.5% HC; 2.5% TDZ; 50 g/L KNO_3 + 2% Oil; 50 g/L KNO_3. HC = hydrogen cyanamide; TDZ = thidiazuron Means with the same letter are not significantly different. ns = no significant difference.

Treatment	Individual Kernel				Percentage of Kernels	
	Width (mm)	Thickness (mm)	Colour	Roughness	Double	Shrivelled
Control	11.34 ns	7.75 ns	2.47 a	1.54 ns	0.09 ns	0.47 ns
HC + Oil	11.11	7.67	2.47 a	1.57	0.00	0.56
Oil	11.13	7.59	2.43 ab	1.62	0.06	0.70
HC	11.03	7.66	2.36 abc	1.58	0.00	0.61
TDZ	11.25	7.64	2.39 abc	1.62	0.00	0.45
KNO_3 + Oil	11.07	7.68	2.27 c	1.55	0.08	0.45
KNO_3	11.38	7.73	2.28 bc	1.54	0.00	0.16
Significance level	*0.0520*	*0.8431*	***0.0404***	*0.3634*	*0.6816*	*0.4461*
LSD 5%	-	-	*0.15*	-	-	-

Table 11. The average growth index in terms of all secondary growth, secondary sylleptic shoot growth, secondary long shoot growth, secondary short shoot growth and secondary spur growth for 'Independence' almond trees in Worcester during the 2019/2020 season. Treatment concentrations were as follow: 0.5% HC + 2% Oil; 2% Oil; 0.5% HC; 2.5% TDZ; 50 g/L KNO_3 + 2% Oil; 50 g/L KNO_3. HC = hydrogen cyanamide; TDZ = thidiazuron Means with the same letter are not significantly different. ns = no significant difference.

Treatment	Growth index				
	All secondary growth	Secondary shoots with sylleptic growth	Secondary long shoot	Secondary short shoot	Secondary spur
Control	0.85 a	0.05 ns	0.06 ns	0.65 ab	0.10 c
HC + Oil	0.48 b	0.00	0.00	0.26 c	0.22 a
Oil	0.64 ab	0.04	0.03	0.41 bc	0.17 b
HC	0.49 b	0.01	0.01	0.31 c	0.16 b
TDZ	0.54 b	0.04	0.00	0.31 c	0.19 ab
KNO_3 + Oil	0.89 a	0.02	0.02	0.68 a	0.18 ab
KNO_3	0.65 ab	0.04	0.08	0.47 abc	0.06 c
Significance level	***0.0061***	*0.4732*	*0.2093*	***0.0070***	***<0.0001***
LSD 5%	*0.26*	-	-	*0.26*	*0.04*

Table 12. The effect of different rest breaking treatments and primary shoot type on the average number of secondary sylleptic, long and short shoots formed at the Hex Poort (Worcester) 'Independence' trial site during the 2019/2020 season. Treatment concentrations were as follow: 0.5% HC + 2% Oil; 2% Oil; 0.5% HC; 2.5% TDZ; 50 g/L KNO_3 + 2% Oil; 50 g/L KNO_3. HC = hydrogen cyanamide; TDZ = thidiazuron Means with the same letter are not significantly different. ns = no significant difference.

Treatment	Average number of secondary growth		
	Sylleptic shoots	Long shoots	Short shoots
Rest breaking treatment			
Control	0.20 ns	0.30 ns	9.95 ab
HC + Oil	0.00	0.00	4.55 d
Oil	0.15	0.15	7.65 bcd
HC	0.05	0.05	5.50 cd
TDZ	0.15	0.00	5.30 cd
KNO_3 + Oil	0.10	0.10	11.55 a
KNO_3	0.15	0.40	8.75 abc
Primary shoot type			
Long shoot	0.23 a	0.21 ns	10.86 a
Short shoot	0.00 b	0.07	4.36 b
Significance level			
Treatment	*0.5572*	*0.1909*	***0.0004***
Primary shoot type	***0.0001***	*0.1379*	***<0.0001***
*Treatment*Primary shoot type*	*0.5572*	*0.4070*	*0.4914*
LSD 5%			
Treatment	-	-	*3.46*
Primary shoot type	*0.11*	-	*1.85*
*Treatment*Primary shoot type*	-	-	-

Table 13. The effect of different rest breaking treatments and primary shoot type interaction on the average number of secondary spurs formed at the Hex Poort (Worcester) 'Independence' trial site during the 2019/2020 season. Treatment concentrations were as follow: 0.5% HC + 2% Oil; 2% Oil; 0.5% HC; 2.5% TDZ; 50 g/L KNO_3 + 2% Oil; 50 g/L KNO_3. HC = hydrogen cyanamide; TDZ = thidiazuron Means with the same letter are not significantly different.

Treatment combination		Average number of secondary spurs	
Control	Long shoot	12.0	de
	Short shoot	5.2	gh
HC + Oil	Long shoot	27.0	a
	Short shoot	10.7	def
Oil	Long shoot	22.4	abc
	Short shoot	7.4	efgh
HC	Long shoot	18.5	c
	Short shoot	9.9	defg
TDZ	Long shoot	21.5	cd
	Short shoot	12.4	d
KNO_3 + Oil	Long shoot	23.9	ab
	Short shoot	8.2	defgh
KNO_3	Long shoot	7.2	fgh
	Short shoot	4.5	h
Significance level			
Treatment		***<0.0001***	
Primary shoot type		***<0.0001***	
*Treatment*Primary shoot type*		***0.0004***	
LSD 5%			
Treatment		*3.42*	
Primary shoot type		*1.83*	
*Treatment*Primary shoot type*		*4.80*	

Table 14. Effect of different rest breaking agents on the reproductive bud break (BB), vegetative BB and total BB as a percentage of the total number of dormant buds, as well as fruit set of the 'Independence' almond orchard at Groenrivier (Riebeek West) during the 2020 spring. Treatment concentrations were as follow: 0.5% HC + 2% Oil; 2% Oil; 0.5% HC; 2.5% TDZ; 50 g/L KNO_3 + 2% Oil; 50 g/L KNO_3. HC = hydrogen cyanamide; TDZ = thidiazuron; BB = bud break; BBveg@maxBBrep = Vegetative BB at maximum reproductive BB. Means, within each column, with the same letter are not significantly different. ns = no significant difference.

Treatment	Reproductive BB			Vegetative BB				Total BB		
	Onset *(days)	Maximum (%)	BB period (days)	Onset *(days)	BB_{veg} @ max BB_{rep} (%)	Maximum (%)	BB period (days)	Onset *(days)	Maximum (%)	BB period (days)
Control	11.05 ns	39.76 ns	25.65 ns	32.88 a	12.23 cd	47.12 b	24.52 c	10.93 a	86.99 b	46.47 ns
HC + Oil	7.97	43.33	22.43	10.01 c	32.99 a	48.70 b	40.79 a	6.42 d	92.03 a	44.38
Oil	9.35	39.42	24.85	12.15 c	31.42 a	50.67 ab	43.15 a	8.69 bc	90.08 ab	45.51
HC	10.79	37.90	25.31	20.70 b	20.25 bc	49.24 b	30.31 ab	10.49 ab	87.13 b	46.51
TDZ	10.26	42.51	23.54	12.51 c	28.54 ab	47.41 b	42.89 a	9.29 abc	89.93 ab	46.11
KNO_3 + Oil	8.23	39.44	22.77	11.31 c	33.65 a	53.47 a	40.79 a	7.47 cd	92.91 a	44.63
KNO_3	10.24	39.18	21.76	23.83 b	9.70 d	49.81 ab	31.37 bc	9.97 ab	88.99 ab	45.23
Significance level	*0.0984*	*0.1036*	*0.5508*	***<0.0001***	***<0.0001***	***0.0193***	***<0.0001***	***0.0007***	***0.0311***	*0.9437*
LSD %	-	-	-	*5.73*	*8.68*	*3.66*	*7.12*	*2.15*	*4.01*	-

* days to 5% BB from application of rest breaking treatment

Table 15. Effect of different rest breaking treatments and shoot type on the average percentage fruit set at the Groenrivier (Riebeek West) 'Independence' trial site during the 2020/2021 season. Treatment concentrations were as follow: 0.5% HC + 2% Oil; 2% Oil; 0.5% HC; 2.5% TDZ; 50 g/L KNO_3 + 2% Oil; 50 g/L KNO_3. HC = hydrogen cyanamide; TDZ = thidiazuron. Means, within each column, with the same letter are not significantly different. ns = no significant difference.

Treatment	Average percentage fruit set	
Rest breaking treatment		
Control	17.23	ns
HC + Oil	13.97	
Oil	13.21	
HC	15.59	
TDZ	16.27	
KNO_3 + Oil	13.09	
KNO_3	15.31	
Shoot type		
Long shoot	10.48	c
Short shoot	12.99	b
Spur	21.34	a
Significance level		
Treatment	*0.0955*	
Shoot type	***<0.0001***	
*Treatment*Shoot type*	*0.6520*	
LSD 5%		
Treatment	-	
Shoot type	*2.16*	
*Treatment*Shoot type*	-	

Table 16. Effect of different rest breaking agents on the reproductive bud break (BB), vegetative BB and total BB as a percentage of the total number of dormant buds, as well as fruit set of the 'Independence' almond orchard at Hex Poort, (Worcester) during the 2020 spring. Treatment concentrations were as follow: 0.5% HC + 2% Oil; 2% Oil; 0.5% HC; 2.5% TDZ; 50 g/L KNO_3 + 2% Oil; 50 g/L KNO_3. HC = hydrogen cyanamide; TDZ = thidiazuron; BB = bud break; BBveg@maxBBrep = Vegetative BB at maximum reproductive BB. Means, within each column, with the same letter are not significantly different. ns = no significant difference.

Treatment	Reproductive BB			Vegetative BB				Total BB		
	Onset *(days)	Maximum (%)	BB period (days)	Onset *(days)	BB_{veg} @ max BB_{rep} (%)	Maximum (%)	BB period (days)	Onset *(days)	Maximum (%)	BB period (days)
Control	10.51 ns	41.07 ns	23.99 ns	34.88 ab	9.79 ns	35.91 ns	25.22 c	10.51 ns	76.99 ns	49.59 ns
HC + Oil	9.48	44.06	18.72	19.74 de	13.19	28.60	36.36 ab	9.31	72.56	46.80
Oil	10.72	42.76	24.28	29.00 bcd	15.52	34.12	30.67 bc	10.61	76.40	49.06
HC	9.06	43.35	25.24	27.12 cd	8.56	31.96	31.88 abc	9.06	75.32	49.94
TDZ	9.26	38.03	26.85	21.75 de	17.20	37.75	40.03 a	9.10	75.78	52.68
KNO_3 + Oil	10.52	43.02	22.38	30.28 abc	6.62	28.53	31.72 abc	10.50	71.54	51.51
KNO_3	9.21	39.59	28.29	37.38 a	5.85	37.27	26.72 c	9.08	76.86	55.02
Significance level	*0.7994*	*0.5593*	*0.1987*	***<0.0001***	*0.1004*	*0.1681*	***0.0120***	*0.7788*	*0.6938*	*0.1962*
LSD %	-	-	-	*7.33*	-	-	*8.57*	-	-	-

* days to 5% BB from application of rest breaking treatment

Table 17. Effect of different rest breaking treatments and shoot type on the average percentage fruit set at the Hex Poort (Worcester) 'Independence' trial site during the 2020/2021 season. Treatment concentrations were as follow: 0.5% HC + 2% Oil; 2% Oil; 0.5% HC; 2.5% TDZ; 50 g/L KNO_3 + 2% Oil; 50 g/L KNO_3. HC = hydrogen cyanamide; TDZ = thidiazuron. Means, within each column, with the same letter are not significantly different. ns = no significant difference.

Treatment	Average percentage fruit set	
Rest breaking treatment		
Control	23.16	ns
HC + Oil	32.10	
Oil	23.69	
HC	29.48	
TDZ	31.48	
KNO_3 + Oil	32.60	
KNO_3	25.16	
Shoot type		
Short shoot	15.88	b
Spur	40.63	a
Significance level		
Treatment	*0.4250*	
Shoot type	***<0.0001***	
*Treatment*Shoot type*	*0.4331*	
LSD 5%		
Treatment	-	
Shoot type	*6.35*	
*Treatment*Shoot type*	-	

PAPER 3: Investigating flower morphology, pollination and the role of bees in self-compatible 'Independence' almond trees

Additional index words. Bearing position, epistigmatic flower, autogamy, pollen vector

---

Abstract.

Successful fertilization is required for the production of an almond crop. Most commercial almond cultivars are self-incompatible and therefore rely on pollinators, such as honeybees, for cross-pollination and subsequent fertilization. This holds financial implications such as ceding a part of the bearing capacity to a compatible cross-pollinator cultivar, as well as requiring commercial pollination services. Cross-pollination can also be a limiting factor for almond production due to unfavourable weather for bee activity during flowering. To address these problems, almond breeding programmes have shifted their focus to later flowering, self-compatible cultivars, such as Independence and Soletta. The purpose of this study was to determine the effect of commercial beehives on the fruit set, yield and quality of 'Independence' almonds, as well as the effect of a nearby cross-pollinator ('Nonpareil') on these parameters. 'Independence' almond flower morphology was examined, with specific reference to the style to stamen length ratio, and to determine the effect of bearing positions on flower quality. Fruit set was 10% to 20% higher, in the presence of bees, compared to exclusion of bees as pollinators. This translated to a significant increase in yield, however, kernel size was reduced. No significant differences were seen in the post-harvest quality parameters. The presence of a nearby cross-pollinator ('Nonpareil') had no effect on yield, nor post-harvest quality parameters in 'Independence' almonds. A higher quality flower was observed on spurs, compared to other bearing positions, and could explain spurs having a higher percentage fruit set. When considering the style to stamen length ratio, epistigmatic flowers were observed in 'Independence' almond trees. Collectively, these results show that the epistigmatic flowers of the self-compatible almond cultivar, 'Independence', have the capacity for successful autogamy, however, the use of pollinators such as bees is necessary to maximise

yield. Furthermore, the presence of a compatible cross-pollinator ('Nonpareil') did not have an effect on the production and nut quality of 'Independence'.

In most deciduous fruit trees, fruit set is dependent on fertilization. This requires the transfer of compatible pollen to the stigma, followed by the germination of the pollen and subsequent growth of the pollen tube down the style to the ovule, and finally, the successful fertilization of the egg cell and two polar nuclei (Stösser et al., 1996). Commercial almond cultivars are predominantly self-incompatible (Socias i Company, 1990), a genetically inherited trait that promotes pollination by another genetically different individual of the same species, by preventing self-fertilization (Taiz et al., 2018) via the incompatibility/rejection of own pollen by the stigma. Thus, obtaining a commercially viable crop from self-incompatible fruit trees requires a compatible cross-pollinator species with an overlapping flowering period within the same or nearby orchard to allow for allogamy (cross-fertilization). Furthermore, to assure pollen transfer among such cultivars pollen vectors, such as wind or insects, are required. Honeybees (*Apis mellifera* L.) play an essential role by acting as vectors thus ensuring agricultural diversity and production (Aizen et al., 2009; Lee et al., 2018). According to Aizen et al. (2008) the dependence of crop production on pollinators have increased significantly since 1961 compared to non-dependent crops in both developed and developing countries, increasing the demand for commercial pollination services. This could be of great economic importance, as the global contribution of pollinators to the production of food directly consumed by humans, had an estimated value of €153 billion in 2005 (Gallai et al. 2009).

Almonds are traditionally one of the earliest blooming temperate fruit crops, flowering when the climatic conditions are not always optimal for pollination via insects such as honeybees, thus the need for cross-pollination can be a key limiting factor for almond production. Almond breeding programmes have therefore shifted their focus to the development of later flowering cultivars that bloom in more favourable climatic conditions, as well as self-compatible almond cultivars that are less dependent on pollinators (Bernad and Socias i Company, 1995; Socias i Company, 1990, Vargas et al., 1997). A successful self-compatible almond cultivar is characterised by similar pollen tube growth, fruit set and commercial crop load following self-pollination (autogamy or geitonogamy), compared to that achieved after cross-pollination with compatible pollen (allogamy), under orchard condition

(Socias i Company et al., 2004). Self-compatibility of almonds was introduced by the European breeding programmes mostly through cultivars from the Puglia almond populations in Italy, whereas the Californian programmes focused on peach as the self-compatible source (Socias i Company, 2017; Socias i Company et al., 2011). 'Independence' (Patent no. 20295), a fully self-compatible almond cultivar, was released by Zaiger's Inc. Genetics and originated from a cross between the All-In-One almond cultivar and Almond selection 2168 (Batlle et al., 2017).

'Independence' as a later flowering, self-compatible cultivar presents potential financial benefits for growers as it is does not require a cross pollinator and has a reduced dependency on bees for pollination, while some marketers have created the impression of complete bee-independence for this cultivar (Doll, 2012; Mercer, 2014. This effectively increases the bearing capacity for the preferred cultivar (Cape Almonds, 2017). Batlle et al. (2017) described 'Independence' as "a prolific bloomer and excellent producer", with other characteristic such as high kernel quality, large size and light colour. 'Independence' quickly gained popularity in the United States of America, increasing from 16 ha planted in 2008 after its release, to almost 2000 ha in just ten years (California Department of Food and Agriculture, 2019). In the South African context, Zaiger's Inc. Genetics and Zaiger SA granted ZZ2 (Cape Almonds) the exclusive master-license to cultivate 'Independence' in Southern Africa in 2016. Currently Cape Almonds, in partnership with several sub-licensee growers, envisage to plant, 689 ha of 'Independence' almond trees throughout the Western Cape, South Africa, by 2021 (personal communication, Cape Almonds).

To ensure a commercially viable yield in almond production, a high flowering density followed by adequate pollination is necessary for acceptable fruit set (Kodad and Socias i Company, 2006). Flower quality is influenced by the different bearing positions of flowers and subsequently affects fruit quality, as seen in apple (De Silva et al., 2000; Lauri et al., 1996), peach (Bruchou and Genard, 1999) and almond (Kodad and Socias i Company, 2006). Kodad and Socias i Company (2006) found a significant reduction in kernel size of almonds borne on spurs compared to other shoot types. Flower quality depends on many factors such as flower size, pistil development, stigma receptivity and ovule longevity (Williams, 1965). Some authors have argued that the relative position of the receptive stigma and dehiscing anthers could determine the capacity for natural self-pollination in self-compatible cultivars (Bernad and Socias i Company, 1995; Weinbaum, 1985). Successful self-pollination of self-compatible almond, in the absence of pollinators, have been ascribed to a short style length presenting the stigma below the anthers (Vasilakakis and Porlingis, 1984).

The objective of this study was to determine the effect of commercial beehives and cross-pollinator presence on the fruit set and nut quality of the self-compatible 'Independence' almond cultivar under South African conditions. In relation to this, flower quality was also investigated by comparing anther and style morphology from different bearing positions.

Materials and Methods

Trial 1: Flower morphology.

Plant material and site description. The flower morphology of five- and three-year-old 'Independence' almond trees, was investigated during the spring of 2020. Flowers were sourced from two commercial farms in the Western Cape, South Africa, viz. Tamarak (32°48'53.9"S 18°39'04.1"E; 580 m.a.s.l.) on the mountain near Piketberg and Groenrivier (33°20'45.3"S 18°51'52.2"E; 193 m.a.s.l) near Riebeek West. The 'Independence' trees on 'Viking' rootstocks were planted in 2015 at a 7 x 4 m spacing at the Tamarak trial site, and in 2017 at a 6 x 4 m spacing at the Groenrivier trial site. Both orchards received standard commercial cultivation practices, producing the first commercial yield in February 2018 for the Piketberg trial site and February 2020 for the Riebeek West trial site.

Trial lay-out and data collection. To investigate the flower morphology, a randomised design was used to select thirty single tree replicates. During full bloom (80% open flowers) three flowers were randomly selected from three different bearing positions: approximately in the middle of long shoots (longer than 20 cm), short shoots (3 to 20 cm) and spurs (shorter than 3 cm) at the Piketberg (27 Augustus 2020) and Riebeek West (10 September 2020) trial sites. The flowers from both sites were brought to the laboratory for non-destructive and destructive analysis. Each flower replicate was weighed upon arrival using a Precisa balance (XT 120A, Dietikon, Switzerland). The length of the style was determined by measuring from the top of the ovary to the stigma. Likewise, the length of the stamen was determined by measuring two stamens per stamen whorl (two whorls per flower) from the attachment to the sepal to the anther (Fig. 1.1). Measurements were made using a stereo-microscope (Leika KL 200 LED, Heerbrugg, Switzerland) at a 0.8x enlargement and images were captured using Leica Application Suite Software (Version 3.70, Leica Microsystems Limited, Heerburgg, Switzerland). The dimension of the style relative to the stamen was expressed as the ratio of the two measured variables.

Trial 2: Effect of bees on fruit set and nut quality.

Plant material and site description. During the spring of 2019 and 2020, trials were conducted on 'Independence' almond orchards on the farm Groenrivier (33°20'45.3"S 18°51'52.2"E) in Riebeek West, Western Cape, South-Africa. Trees on 'Viking' rootstock were planted at a 6 x 4 m spacing. Trees used during the 2019 trial were planted in 2016, while two trial sites were used during the 2020 trials, with trees planted in 2017 (Trial site A) and 2016 (Trial site B). An additional 'Independence' orchard, planted in 2017, was included in the 2020 trial to increase the sample size. Temperature data was obtained by placing a Tinytag Plus 2 data logger (Gemini Data Loggers, UK) in each orchard recording the hourly temperature for the duration of the trial. Standard commercial cultivation practices were applied, and each orchard produced its first commercial yield in February 2019 (four-year-old trees) and 2020 (three-year-old trees), respectively. During both seasons, two beehives per hectare were placed in the orchards at 10% flowering and remained for the duration of the flowering period, as per commercial practice.

Trial lay-out, treatment application and data collection. In 2019, the effect of commercial beehives on fruit set, yield and post-harvest quality was investigated by using a randomised design to select eight single tree replicates. On each tree, four branches were randomly selected and tagged. Two of the branches were simple structures, consisting of a single, one-year-old shoot on two-year-old wood, and two complex structures, which included two or more one-year-old shoots, including sylleptic growth, on two-year-old wood. Enclosures covering the whole tree were made from 40% black shade netting (Knittex (Pty) Ltd, Brackenfell, Western Cape), and covered eight of the trees to exclude contact with bees as pollinators (enclosed trees). The remaining eight trees were left uncovered to serve as controls. The enclosures were placed in the orchard at the first signs of bud break and removed six weeks later when all flowers were at the "Petal fall" stage (Stage G) as per scale notation invented by Felipe in 1977 (Thomas and Connell, 2018). Bee activity was monitored at 10:00 am twice during the flowering period by walking down a row at a steady pace for two minutes, passing approximately 29 trees, observing the flowers, and counting the number of bees present. No bees were observed inside the enclosed structures. To determine if the shade netting had an effect on the temperature inside the enclosures, Tinytag Plus 2 temperature loggers (Gemini Data Loggers, UK) were placed inside one enclosure and the hourly temperatures were compared to temperatures recorded by a second Tinytag in the open orchard. In addition, a Licor light meter (LI-250, Lincoln, NE) was used to compare the irradiance inside the

enclosures versus the open orchard. The total number of open flowers on each tagged branch was counted at full bloom (80% open flowers). Six weeks after full bloom, the number of fruitlets present on each branch was counted and fruit set was determined as the percentage fruitlets present divided by the initial number of flowers on each branch.

The fruit were harvested commercially on 30 and 31 January 2020. Yield and post-harvest quality data were determined in the same manner as described in Paper 2.

In 2020, the approach was different, two sets of fifteen single tree replicates (Trial site A and B, respectively) were used. During the "Popcorn" stage, Stage D2 as per scale notation invented by Felipe in 1977 (Thomas and Connell, 2018), four one-year-old shoots (10 to 30 cm) were selected per tree and all the reproductive buds were counted per shoot. Any open flowers on the shoots were removed and white paper bags were used to cover two of the shoots per tree while two shoots were left uncovered to serve as controls. The bags were removed after ten days and all unopen flowers were removed (covered and controls). The total number of flowers were then recalculated by subtracting the number of removed, unopen flowers from the initial number of reproductive buds counted on each shoot. Fruit set was determined in a similar manner to that of the previous season. Tinytag Plus 2 temperature loggers (Gemini Data Loggers, UK) were used to compare the temperature inside the bags to that of the open air. Data for the 2021 harvest will not be included in this study.

Trial 3: Effect of a cross-pollination on fruit set and nut quality.

Plant material and site description. During the spring of 2019 and 2020, trials were conducted on three- and four-year-old 'Independence' almond orchards on the farm Hex Poort (33°35'00.4"S 19°29'57.1"E) in Worcester, Western Cape, South-Africa. Trees on 'Viking' rootstock were planted in 2016 at a 6 x 4 m spacing. Temperature data was obtained by placing a Tinytag Plus 2 data logger (Gemini Data Loggers, UK) in the orchard recording the hourly temperature for the duration of the trial. Standard commercial cultivation practices were applied, and each orchard produced the first commercial crop in February 2019. The orchards were located in the mountain near natural fynbos with many bees occurring naturally in this orchard, therefore no additional commercial beehives were placed in the orchard by the grower.

Trial lay-out, treatment application and data collection. To determine if the presence of a cross-pollinator has an effect on the fruit set, yield and quality, three different hand-pollination treatments were applied to ten, single tree replicates that were selected using a

randomised design. The treatments consisted of hand-pollination of 'Independence' flowers with 'Independence' flowers from a different orchard; hand-pollination of 'Independence' flowers with 'Nonpareil' flowers; and no hand-pollination (untreated control). Four scaffold branches per tree were randomly selected, of which two branches were simple structures, consisting of a single, one-year-old shoot on two-year-old wood, and two were complex structure, including two or more one one-year-old shoots with sylleptic growth, on two-year-old wood. Open flowers from nearby 'Independence' and 'Nonpareil' orchards were collected and used to hand-pollinate all the flowers on the tagged branches of ten trees each when the 'Independence' trial orchard was at 50% (2 September 2019) and 80% (6 September 2019) full bloom in the first season, and at 70% (5 September 2020) and 90% (9 September 2020) full bloom in the second season. Each collected flower was dabbed lightly on two of the flowers receiving hand-pollination, ensuring that pollen is secured on the stigma. This procedure was repeted until all the flowers on the tagged branches received hand-pollination. The ten control trees received no hand pollination. The total number of open flowers on each branch was recorded.

Six weeks after full bloom, the number of fruitlets present on each branch was counted and fruit set was determined by dividing the total number of fruitlets by the total number of flowers counted on each branch and was expressed as percentage fruit set. The nuts from the four branches were harvested on 11 and 12 February 2020, during commercial harvest. The fruit were dried for approximately three days until a moisture content of 6% was reached, after which the individual parts were weighed per branch (dry weight, in-shell weight and kernel weight) as described for Trail 2. After harvest, the branch diameters were measured and used to calculate the branch cross-sectional area (BCSA). Yield efficiency ($g \cdot cm^{-2}$) was determined by dividing the yield (g) of each branch by the BCSA, as well as the branch nut density (number of buds per cm^{-2}) by dividing the number of fruit by the BCSA. Post-harvest quality parameters (kernel weight, length, width and thickness, pellicle colour and roughness) of all the nuts were conducted as described for Trial 2. The 2021 harvest data will not be included in this study.

Statistical analysis. The data from all three the trials were analysed using the linear model procedure and when the F-statistic indicated significant differences at a 5% level, Fishers LSD test was performed as a post hoc test in SAS Enterprise Guide 7.1 (SAS Institute Inc., Cary, North Carolina, USA).

Results

Trial 1: Flower morphology.

The average style length ± standard error was 11.4 ± 0.2 mm, average stamen (filament plus anther) length 7.3 ± 0.1 mm for the outer whorl, and average inner stamen whorl length 5.3 ± 0.1 mm in 'Independence' flowers sourced from the commercial farm in Piketberg. The flowers from the Riebeek West region had an average style length of 11.0 ± 0.1 mm, average outer whorl stamen length of 7.2 ± 0.1 mm, and an average stamen length of 5.4 ± 0.1 mm for the inner whorl.

Significant differences were found among different bearing positions in average flower weight for the orchard in Piketberg (p=0.003) and Riebeek West (p=0.0002) (Table 1.1 and 1.2). A higher average flower weight was found in flowers borne on spurs (329.7 mg), compared to the long shoots and short shoots, with the latter having the lowest average flower weight (281.9 mg) at Piketberg, albeit not significantly lower than those on long shoots. The orchard in Riebeek West also had the highest average flower weight for flowers borne on spurs (282.2 mg), but these did not differ significantly from that on the long shoots. The short shoots had a significantly lower average flower weight (231.9 mg) compared to the other two bearing positions.

The average style length did not differ significantly among bearing positions for the orchard in Piketberg, however, flowers borne on spurs (11.5 mm) and long shoots (11.4) had significantly longer styles (p<0.0001) compared to flowers sourced from short shoots (10.2 mm) for the orchard in Riebeek West (Table 1.1 and 1.2).

Significant differences were found in the stamen length of the outer whorl among different bearing positions for both the orchard in Piketberg (p<0.0001) and Riebeek West (p=0.0007) (Table 1.1 and 1.2). At the Piketberg farm, stamen length (7.9 mm) was significantly longer in flowers borne on spurs compared to those on both short (7.1 mm) and long shoots (6.8 mm), the latter not differing significantly from each other. At the orchard in Riebeek West, flowers borne on short shoots had a significantly shorter outer stamens (6.8 mm) compared to that of flowers borne on long shoots (7.3 mm) and spurs (7.5 mm), the latter, again, not differing significantly from each other. Likewise, significant differences were found for the stamen length of the inner whorl among different bearing positions for both orchards

(p<0.0001) (Table 1.1 and 1.2). At the Piketberg orchard, spurs once again had significantly longer inner stamens (5.8 mm) compared to the other bearing positions, while stamen lengths of flowers borne on short and long shoots (5.1 and 5.0 mm, respectively) did not differ significantly from each other. At the Riebeek West orchard, flowers borne on spurs had significantly longer inner stamens (5.8 mm) compared to the other bearing positions, while flowers borne on short shoots had significantly shorter stamens (5.0 mm) compared to the other two bearing positions.

The style to stamen length ratio of the outer whorl, significantly differed between bearing positions for the orchard in Piketberg (p<0.0001) (Table 1.1). The long shoots had a significantly higher ratio (1.78), followed by short shoots (1.59) and the lowest ratio was found in (1.41). In the Riebeek West orchard, no significant differences were found in the style to stamen ratio for the outer whorl (Table 1.2). Likewise, significant differences were found among bearing positions for the style to stamen ratio for the inner whorl in the Piketberg orchard (p<0.0001), but not for the orchard in Riebeek West (Table 1.1 and 1.2). At the Piketberg orchard, flowers borne on long shoots had a significantly higher ratio (2.44) followed by short shoots (2.20) and the lowest ratio was found in spurs (1.93).

Trial 2: Effect of bees on fruit set and nut quality.

An average of seven bees were observed in a two-minute period during the spring of 2019 and five bees during the spring of 2020. The shade netting and the paper bags had little to no effect on the temperature inside the structures (shade net or paper bags) as seen in Fig. 2. The shade nets reduced the irradiation inside the structures by 56%, to 1127.5 µmol.m^{-2}.s^{-1}, compared to the 2005.0 µmol.m^{-2}.s^{-1} outside of the structures. Measurements were taken at 13:45 on a clear, cloudless day. Therefore, the use of shade netting to exclude bees as pollinators was not used during the second year's trial.

The average percentage fruit set, was significantly decreased from 43.6% to 23.4% in trees when bees were excluded during bloom in the first season (p<0.0001), as well as from 25.5% to 6% for the trees at Trial site A in the second season (p=0.002), compared to trees subject to commercial beehive activity. However, no significant difference was found between these treatments for the trees at Trial site B in the second season (Table 2.1 and 3).

The open trees exposed to commercial beehives had higher dry weight (7.9 kg), in-shell weight (3.7 kg) and kernel weight (2.6 kg) per tree, as well as yield efficiency (29.57 g·cm^{-2})

compared to the enclosed trees with 2.1, 0.99, 0.6 kg and 12.4 g·cm^{-2}, respectively (Table 2.1). However, no significant difference was observed in the percentage kernel between trees exposed to commercial beehives, and those covered in shade netting.

Individual kernel weight, length and width was lower in trees not covered in shade netting while those from trees covered in shade netting produced significantly bigger kernels, with the weight (1.3 g), length (25.1 mm) and width (12.6 mm) of individual kernels compared to the average individual kernel weight (1.0 g), length (22.9 mm) and width (11.4 mm) produced by trees that were subject to commercial beehives. Individual kernel thickness, pellicle colour, or roughness, and percentage double or shrivelled kernels did not differ between the treatments (Table 2.2 and 2.3).

Trial 3: Effect of a cross-pollinator on fruit set and nut quality.

In both seasons, hand pollination with own or 'Nonpareil' pollen did not improve fruit set compared to open, untreated control flowers (Table 4.1 and 5). The fruit set was generally lower in the second season. No significant differences were found in the yield per branch, percentage kernel, branch yield efficiency and nut density, as well as any of the post-harvest quality parameters for any of the hand pollination treatments, compared to the untreated control (Table 4.1 and 4.2).

Discussion

The average style and stamen lengths indicate that flowers of the self-compatible 'Independence' almond cultivar are epistigmatic, with stigma length exceeding that of the stamens, and the stigma thus protruding above the anthers (Fig. 1.2). Kumar and Kumar (2000) observed epstigmatic flowers in eleven of the sixteen self-compatible almond cultivars they examined. Earlier work by De Palma and Godini (1994) also support these findings. Kumar and Kumar (2000) found no relationship between self-compatibility and flower morphology in the twenty almond cultivars tested. However, Weinbaum (1985) and Bernad and Socias i Company (1995) suggested that the spatial ratio between the stigma and anthers could be an indication of the capacity for natural autogamy in self-compatible cultivars. These findings were refuted by Godini et al. (1992) who showed no correlation between fruit set and stigma/anther position following unassisted self-pollination. However, these authors still showed a substantial increase in average fruit set following self-pollination when comparing

unassisted (10.4%) and hand-pollination (42.1%). Therefore, they concluded that adequate insect vectors are necessary for optimum self-pollination in self-compatible almond cultivars.

Studies on the influence of bearing positions on almond flower morphology, in particular stamen length, is lacking. In this study, the different bearing positions influenced the flower weight and stamen length of both whorls. When the bearing positions did not influence the stigma length, the ratio of stigma to stamen length was still affected, and *vice versa*. However, the style to stamen length ratio remained larger than one, irrespective of bearing position. This is in accordance with a study done by Pattern et al. (1986) indicating that flower quality can be influenced by bearing position. They reported that reproductive buds of sweet cherries situated on spurs generally start to bloom later than flowers at the base of longer shoots. These flowers were also lower in fresh weight of flowers, pistils, ovary and fruit size, as well as the soluble solid concentration of subsequent fruit, therefore indicating that flowers of a lower quality tend to open later. According to our results, flowers borne on spurs had the highest average weight, and therefore, according to the conclusion of Pattern et al. (1986), a higher quality flower compared to the other bearing positions. Tombesi et al. (2016) found that almond fruit are predominantly borne on spurs. This statement is supported by our results from Paper 2 indicating a higher average fruit set percentage on spurs, compared to long and short shoots. Tombesi et al. (2016) related pistil and ovary fresh weight to flower and fruit quality, while our study unfortunately only examined the style length. There was, however, no direct relationship between style length and bearing position. It should be kept in mind that assimilates from the previous season can also influence the time of anthesis, as well as the flower size, which, in turn, can contribute to variability in flower and fruit quality (Tombesi et al., 2016).

In our study the presence of a cross-pollinator had no effect on the yield and post-harvest quality parameters. This is similar to results from Dicenta et al. (2002) who found no difference in the fruit quality of self-compatible almond genotypes after self and cross-pollination. They concluded that cross-pollinators are not necessary to produce a commercially viable yield in single cultivar orchards consisting of self-compatible almond trees.

Self-incompatible almond orchards require a cross-pollinator, as well as pollen vectors that ensures pollen transfer among cultivars, to produce a feasible yield (Vargas et al., 1997), both having annual financial implications. A shift towards more efficient almond production can be established by means of planting self-compatible cultivars, making self-compatibility

one of the most desired traits in almond production and breeding (Socias i Company, 1990; Socias i Company et al., 1998). When comparing self and cross-pollination of self-compatible almond cultivars, fruit set results have been contradictory. Socias i Company and Felipe (1987) found significant differences in fruit set percentage of the self-compatible almond cultivar, Aylés, after self-pollination and cross-pollination with Selection A-10-8. However, Godini et al. (1994) and Dicenta et al. (2002) showed no difference in average fruit set percentage following self and cross-pollination of self-compatible almond cultivars, which is congruent with results found in this study.

Even though the fruit set did not differ among treatments in both years, the average percentage fruit set was substantially higher during the second season's trial. Water shortage during March and April 2020, just after harvest, could have influenced the irrigation scheduling of this orchard, leading to drought stress in some of the trees during a critical developmental phase. Doll (2017) reports that post-harvest water stress could decrease the following year's crop load to a greater extent than water deficit during pre-harvest conditions due to the negative impact on reproductive bud development in the late season. With that said, a study by Tombesi et al. (2016) has shown that almond yield is less correlated with the number of flowers that set fruit and more correlated with the abundance of flowers on the trees. They reported that an increase in relative fruit set could not compensate for low flower densities. This could serve as a plausible explanation for the increase in average fruit set in the year following deficit irrigation. The Worcester orchard had many bee colonies occurring naturally, due to surrounding fynbos, enhancing successful self-pollination in this orchard. Therefore, the control trees that did not receive hand-pollination treatments, did not differ significantly in percentage fruit set and kernel yield compared to the trees that received assisted pollination, whether from foreign or own pollen.

Self-fertility, together with autonomous self-pollination was the driving force behind the development of the self-compatible almond cultivar, 'Independence' (Socias i Company, 2017). However, little research has been done on the true dependence of self-compatible almond cultivars on bees as pollinators, even though Independence is presented as being a bee-independent cultivar (Doll, 2012; Mercer, 2014). During the 2020/2021 season, the average fruit set percentage increased in both experimental units, but only the 2017 plantings (Trial site A) had a significant increase in fruit set during the presence of bees as pollinators. Even though the increase in fruit set was not significant for the 2016 plantings (Trial site B) (p=0.098), an increased trend of 11.5% was found, compared to the exclusion of bees as pollinators. One

could argue that this increase in fruit set would have been significant at a 10% confidence level, instead of the arbitrary 5% level used in this study. Almonds are a high value crop with local farm prices estimated at R80 per kg kernel and yield potential estimated at 2500 kg per hectare for cultivars such as Nonpareil (Industrial Development Corporation, 2017). The financial benefits of possibly increasing average fruit set with more than 10% would outweigh the cost of hiring commercial beehives for pollination. However, Knight et al. (2006) found that an overestimation of the dependence on pollinators is likely when comparing fruit set between open and isolated trees using individual flowers or inflorescences, due to possible resource reallocation among flowers, as well as across years (Stephenson, 1981; Zimmerman and Pyke, 1988). Sáez et al. (2020) therefore estimated pollinator dependency at the level of entire 'Independence' almond trees and indicated an increase in fruit set percentage and kernel yield per tree of approximately 60% and 20%, respectively, for bee-pollinated trees (five hives per hectare), compared to exclusion of bees. Therefore, these authors concluded that the cultivar is not completely pollinator independent and recommended the use of bees, even in self-compatible almond cultivars, to ensure a maximum yield potential. Paper bags used to exclude bees remained on each selected shoot for ten days, while the photosynthetic ability of the rest of the tree was not influenced. Therefore, bees contributed to the ~300% and ~50% increase in fruit set observed in the 2017 (Trial site A) and 2016 (Trial site B) plantings, respectively.

During the 2019/2020 season, trees subject to bees as pollinators showed ~85% higher fruit set, compared to the enclosed trees. However, the substantially longer period of bee exclusion (approximately four weeks) combined with more than 50% decrease in irradiation under the shade netting most likely resulted in a reduced carbohydrate supply due to reduced photosynthesis induced by the low irradiance. A study by Marchi and Sebastiani (2005) indicated that young peach leaves start to export photosynthetic assimilate from 7-10 days after bud break, corresponding to 32% to 52% of the full leaf expansion, which is congruent with results from sour cherry (*Prunus cerasus* L.) (Kappes and Flore, 1989). Enclosed trees were covered in shade netting well after these dates, therefore limiting the rate of photosynthesis in these trees. As shading has been known to have a thinning effect on peach and apple trees (Byers et al., 1985), the decrease in percentage fruit set was most likely due to decreased irradiance levels under shade net structures. However, the average fruit set percentage for enclosed trees was still 23.37% which is close to the 25% to 40% range that is considered optimal, depending on the cultivar (Kester and Griggs, 1959). Therefore, even in the complete absence of bees as pollinators, combined with photosynthetic limitations, the trees managed to

produce a commercial fruit set percentage close to the acceptable level during the 2019/2020 season. However, trees exposed to commercial beehives and full sun had a substantially higher fruit set during both seasons. These results are congruent with results of a similar study by Sáez et al. (2020) on 'Independence' almond trees in California.

The exclusion of bees as pollinators by shade netting decreased the average yield per tree but had no effect on the percentage kernel, while increasing the individual kernel weight, as well as size during the first season's trial. This is to be expected as a possible thinning action due to shade netting decreased the average set percentage and kernel yield obtained, therefore reducing the number of sinks competing for reserves. No horticultural significant effects were however found for the quality parameters and percentage defects. The increase in nut size is not an economical advantage (Polito et al., 1996) and therefore does not compensate for the decrease in fruit set and yield. It is however important to note that pollinators such as bees can occur naturally in commercial orchard environments and therefore, the addition of commercial beehives would not necessarily lead to the same magnitude of increase in fruit set and yield to the extent shown in these results where bees and other pollinators were totally excluded. This is illustrated by the cross-pollination trial in Worcester where no additional beehives were placed in the orchard, however, the trees that did not received assisted pollination had a similar fruit set and kernel yield compared to trees that received hand-pollination from foreign and own pollen.

Conclusion

Results from these trial show that the self-compatible almond cultivar, 'Independence', have epistigmatic flowers. An average of more than 20% fruit set was obtained, even in the complete absence of bees as pollinators, indicating the capacity for autogamy in this cultivar. With that said, commercial beehives are necessary to ensure a maximum potential yield. From these results it is evident that 'Independence' has reduced the pollinator dependency, but not quite reached complete pollinator independency. Cross-pollination of 'Independence' flowers with pollen from 'Nonpareil' was unable to improve fruit set, yield or nut quality in 'Independence' almond trees.

From the aforementioned findings, we theorise that it is possible to produce a maximum yield for 'Independence' almond trees in the absence of a cross-pollinator, provided that adequate pollen vectors, whether wild or managed, are available to optimise the occurrence of self-pollination.

Literature Cited

Aizen, M.A., L.A. Garibaldi, S.A. Cunningham, and A.M. Klein. 2008. Long-term global trends in crop yield and production reveal no current pollination shortage but increasing pollinator dependency. Curr. Biol. 18:1572-1575.

Aizen, M.A., L.A. Garibaldi, S.A. Cunningham, and A.M. Klein. 2009. How much does agriculture depend on pollinators? Lessons from long-term trends in crop production. Ann. Bot. 103:1579-1588.

Batlle, I., F. Dicenta, R. Socias i Company, T.M. Gradziel, M. Wirthensohn, and F.J. Vargas. 2017. Classical genetics and breeding, p. 111-148. In: R. Socias i Company and T.M. Gradziel (eds.). Almonds: botany, production and uses. CABI, Boston, MA.

Bernad, D. and R. Socias i Company. 1995. Characterization of some self-compatible almonds. II. Flower phenology and morphology. HortScience 30:321-324.

Bruchou, C. and M. Génard. 1999. A space-time model of carbon translocation along a shoot bearing fruits. Ann. Bot. 84:565–576.

Byers, R.E., C.G. Lyons, K.S. Yonder, J.A. Barden, and R.W. Young. 1985. Peach and apple thinning by shading and photosynthetic inhibition. J. Hort. Sci. 60(4):465-472.

California Department of Food and Agriculture. 2019. 2018 California almond acreage report. CDFA, Sacramento, CA, USA.

Cape Almonds. 2017. Prospectus: Independence Cultivar. ZZ2, Mooketsi, South Africa.

De Palma, L. and A. Godini. 1994. Stigma/anther spatial relationship in self-fertile and self-sterile almonds. Acta Hort. 373:161-165.

De Silva, H.N., A.J. Hall, W.M. Cashmor, and D.S. Tustin. 2000. Variation of fruit size and growth within an apple tree and its influence on sampling methods for estimating the parameters of mid-season size distributions. Ann. Bot. 86:493–501.

Dicenta, F., E. Ortega, J.A. Cánovas, and J. Egea. 2002. Self-pollination vs. cross-pollination in almond: pollen tube growth, fruit set and fruit characteristics. Plant Breeding 121:163-167.

Doll, D. 2012. Independence Almond – Some Observations. 7 November 2020. <http://thealmonddoctor.com/2012/06/30/independence-almond-some-observations/>

Gallai, N., J.M. Salles, J. Settele, and B.E. Vaissière. 2009. Economic valuation of the vulnerability of wold agriculture confronted with pollinator decline. Ecol. Econ. 68:810-821.

Godini, A., L. de Palma, and M. Palasciano. 1992. Role of self-pollination and reciprocal stigma/anther position on fruit set of eight self-compatible almonds. HortScience 27(8):887-889.

Godini, A., L. de Palma, and M. Palasciano. 1994. Self-fertile almonds and fruit set by optimized self- and cross-pollination. Acta Hort. 373:157—160.

Industrial Development Corporation. 2017. Study on the market potential for the commercial viability of almond production in South Africa. OABS Development (Pty) Ltd, Paarl, South Africa.

Kappes, E.M. and J.A. Flore. 1989. Phyllotaxy and stage of leaf and fruit development influence initiation and direction of carbohydrate export from sour cherry leaves. J. Amer. Soc. Hort. Sci. 114:642-648.

Kester, D.E. and W.H. Griggs. 1959. Fruit setting in the almond: the effect of cross-pollinating various percentages of flowers. Proc. Amer. Soc. Hort. Sci. 74:206-213.

Knight, T.M., J.A. Steets, and T. Ashman. 2006. A quantitative synthesis of pollen supplementation experiments highlights the contribution of resource reallocation to estimates of pollen limitation. Amer. J. Bot. 93(2):271-277.

Kodad, O. and R. Socias i Company. 2006. Influence of genotype, year and type of fruiting branches on the productive behaviour of almond. Sci. Hortic. 109:297-302.

Kumar, K. and U. Kumar. 2000. Flower morphology versus self-compatibility in almond. Acta Hort. 522:97-102.

Lauri, P.E., E. Térouanne, and J.M. Lespinasse. 1996. Quantitative analysis of relationships between size, bearing-axis size and fruit-set—an apple tree case study. Ann. Bot. 77:277–286.

Lee, H., D.A. Sumner, and A. Champetier. 2018. Pollination markets and the coupled futures of almonds and honey bees: Simulating impacts of shifts in demands and costs. Amer. J. Agr. Econ. 101:230-249.

Marchi, S. and L. Sebastiani. 2005. Sink-source transition in peach leaves during shoot development. J. Amer. Soc. Hort. Sci. 130(6)928-935.

Mercer, D., J. Onulak, and J.M. Fagan. 2014. Alternative pollination methods for California almond trees in response to Colony Collapse Disorder. Date last accessed: 16 December 2020. https://rucore.libraries.rutgers.edu/rutgers-lib/48142/PDF/1/

Pattern, K.D., M.E. Patterson, and E.L. Proebsting. 1986. Factors accounting for the within-tree variation of fruit quality in sweet cherries. J. Amer. Soc. Hort. Sci. 111(3):356-360.

Polito, V.S., W.C. Micke, and D.E. Kester. 1996. Bud development, pollination, and fertilization. Almond production manual. University of California. 3364:98-102.

Sáez, A., M.A. Aizen, S. Medici, M. Viel, E. Villalobos and P. Negri. 2020 Bees increase crop yield in an alleged pollinator-independent almond variety. Nature Research 10:1-7.

Socias i Company, R. 1990. Breeding self-compatible almonds. Plant Breeding Rev. 8:313-338.

Socias i Company, R. 2017. Pollen-style (in)compatibility: development of autogamous cultivars, p. 189-208. In: R. Socias i Company and T.M. Gradziel (eds.). Almonds: botany, production and uses. CABI, Boston, MA.

Socias i Company, R. and A.J. Felipe. 1987. Pollen tube growth and fruit set in self-compatible almond selection. HortScience 22:113-116.

Socias i Company, R., A. J. Felipe, J.G. Aparisi, J.E. García, and F. Dicenta. 1998. The ideotype concept in almond. Acta Hort. 470:51–56.

Socias, R., J.M. Alonso, and J.G. Aparisi. 2004. Fruit set and productivity in almond as related to self-compatibility, flower morphology and bud density. J. Hort. Sci. Biotechnol. 79:754-758.

Socias i Company, R., O. Kodad, J.M. Alonso Segura, J.L. Espada Carbó, P. Chomé, and A. Martínez Treceño. 2011. The introduction of new almond cultivars in Spanish almond growing. Nucis 15:14-17.

Stephenson, A.G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. Annu. Rev. Ecol. Evol. Syst. 12:253-279.

Stösser, R., W. Hartmann, and S.F. Anvari. 1996. General aspects of pollination and fertilization of pome and stone fruit. Acta Hort. 423:15-22.

Taiz, L., G.B. Seymour, and H. Sze. 2018. Gamethophytes, pollination, seeds, and fruits, p. 513-552. In: L. Taiz, E. Zeiger, I.A. Møller, and A. Murphy (eds.). Plant physiology and development. International sixth addition. Oxford Univ. Press, NY, USA.

Thomas, D. and J. Connell. 2018. Assessing phenology of almonds. PIRSA, Adelaide, Australia. Date last Accessed: 29 April 2020. https://2q1ee4456oc52trll42uctl1-wpengine.netdna-ssl.com/wp-content/uploads/2018/08/Assessing-Phenology-in-Almonds-27June-2018.pdf

Tombesi, S., B.D. Lampinen, S. Metcalf, and T.M. DeJong. 2016. Yield in almond is related more to the abundance of flowers than the relative number of flowers that set fruit. Calif. Agric. 71(2):68-74.

Vargas, F.J., J. Clavé, M.A. Romero, I. Batlle, and M. Roviro. 1997. Autogamy studies on almond progenies. Acta Hort. 470:74-81.

Vasilakakis, M.D. and I.C. Porlingis. 1984. Self-compatibility in ‘Truoito’ almond and effect of temperature on selfed and crossed pollen tube growth. HortScience 19:659-661.

Weinbaum, S.A. 1985. Role of natural self-pollination in self-fruitfulness of almond. Sci Hort. 27:295-302.

Williams, R.R. 1965. The effect of summer nitrogen application on the quality of apple blossom. J. Hort. Sci. 40:31-41.

Zimmerman, M. and G.H. Pyke. 1988. Reproduction in *Polemonium*: assessing factors limiting seed set. Am. Nat. 131:723-738.

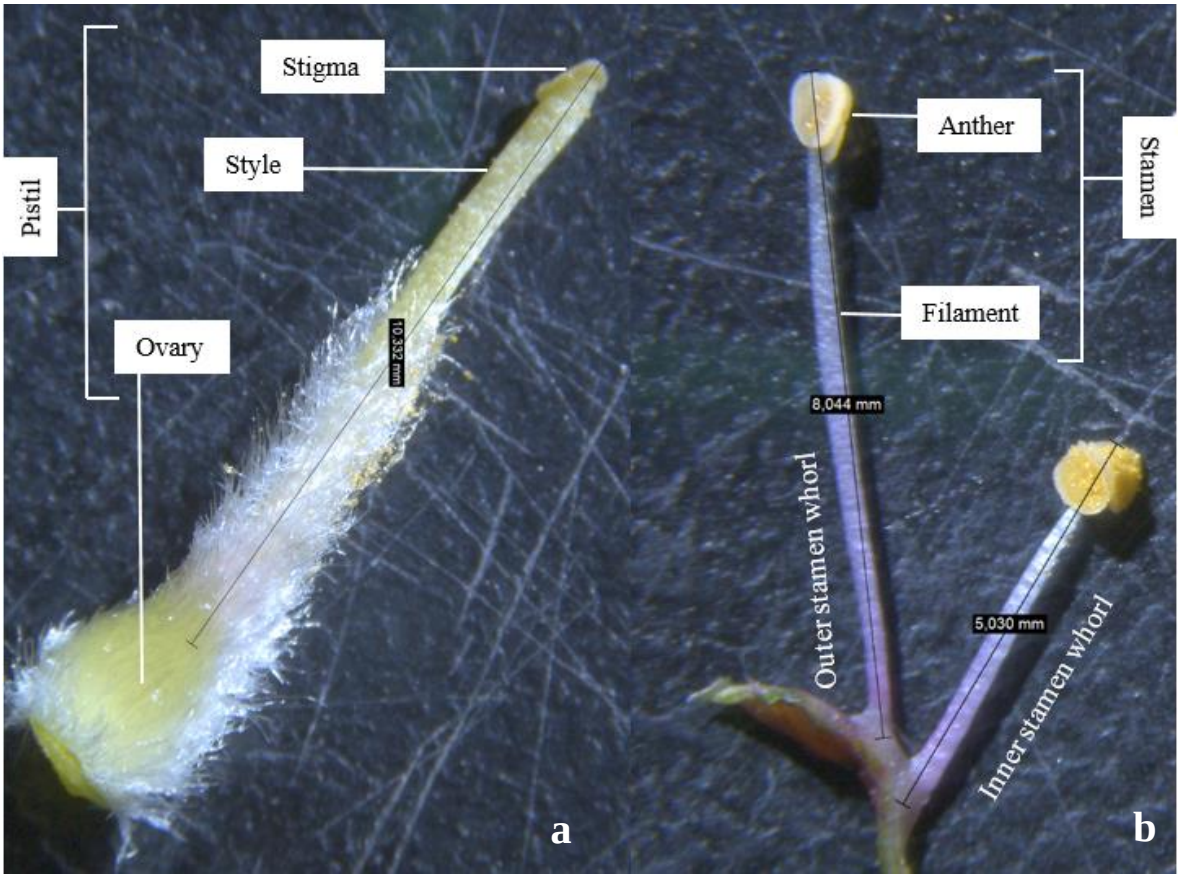


Fig. 1.1. a) Determining style length (mm) by measuring from the top of the ovary to the end of the stigma and b) stamen length (mm) of the inner and outer whorl by measuring from the top of the anther to the start of the sepal in ‘Independence’ almond flowers.

Fig. 1.2. Reproductive organs of an ‘Independence’ almond flower.

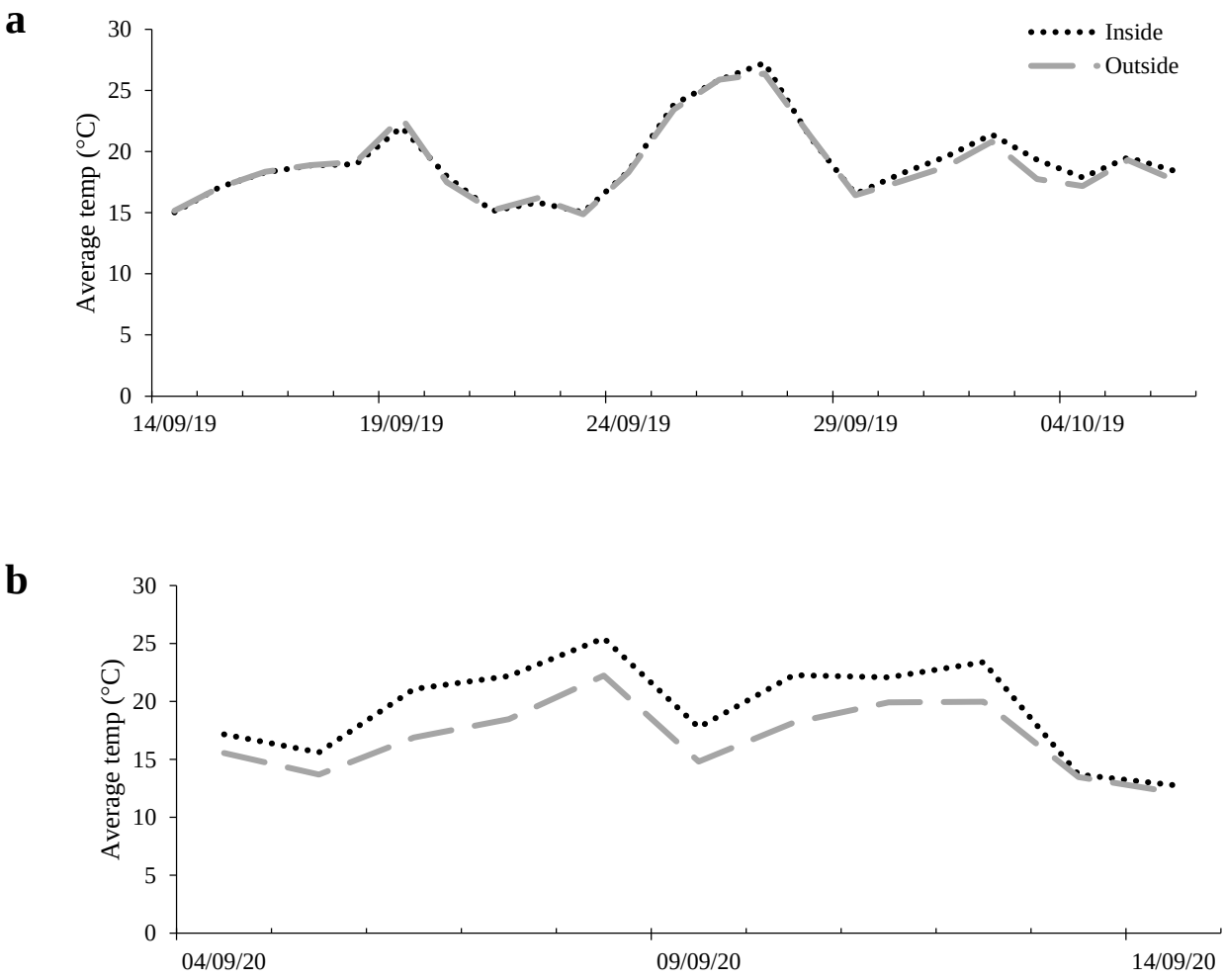


Fig. 2. The daily average temperature (°C) inside and outside of the a) shade net enclosures in used to cover the 'Independence' almond trees during 2019 and b) white paper bags used to cover shoots in 2020 during Trial 2 at Groenrivier, Riebeek West.

Table 1.1. The average flower weight (mg), style length (mm), stamen length (mm) of outer and inner whorl and style to stamen whorl ratios for the three bearing positions of 'Independence' almond trees in Tamarak, Piketberg (2020/2021 season). Means, within each column, with different letters are different at a 5% significance level. ns = no significant difference.

Bearing position	Flower weight (mg)	Style length (mm)	Outer whorl stamen length (mm)	Inner whorl stamen length (mm)	Style: outer whorl stamen length ratio	Style: inner whorl stamen length ratio
Long shoot	295.8 b	11.9 ns	6.8 b	5.0 b	1.78 a	2.44 a
Short shoot	281.9 b	11.2	7.1 b	5.1 b	1.59 b	2.20 b
Spur	329.7 a	11.2	7.9 a	5.8 a	1.41 c	1.93 c
Significance level	***0.0028***	*0.2622*	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***
LSD 5%	*27.5*	-	*0.4*	*0.25*	*0.15*	*0.20*

Table 1.2. The average flower weight (mg), style length (mm), stamen length (mm) of outer and inner whorl and style to stamen whorl ratios for the three bearing positions of 'Independence' almond trees in Groenrivier, Riebeek West (2020/2021 season). Means, within each column, with different letters are different at a 5% significance level. ns = no significant difference.

Bearing position	Flower weight (mg)	Style length (mm)	Outer whorl stamen length (mm)	Inner whorl stamen length (mm)	Style: outer whorl stamen length ratio	Style: inner whorl stamen length ratio
Long shoot	267.8 a	11.4 a	7.3 a	5.5 b	1.56 ns	2.09 ns
Short shoot	231.9 b	10.2 b	6.8 b	5.0 c	1.51	2.09
Spur	282.2 a	11.5 a	7.5 a	5.8 a	1.54	2.00
Significance level	***0.0002***	***<0.0001***	***0.0007***	***<0.0001***	*0.7057*	*0.4261*
LSD 5%	*23.30*	*0.60*	*0.38*	*0.35*	-	-

Table 2.1. Effect of commercial beehives on the percentage fruit set, dry weight, in-shell weight and kernel weight per tree, as well as percentage kernel and yield efficiency of 'Independence' almond trees at Groenrivier, Riebeek West (2019/2020). Means, within each column, with the same letter are not significantly different. ns = no significant difference.

Treatment	Percentage fruit set	Tree dry weight (kg)	Tree in-shell weight (kg)	Tree kernel weight (kg)	Percentage kernel	Yield efficiency ($g.cm^{-2}$)
Bee pollinated (open trees)	43.6 a	7.9 a	3.70 a	2.6 a	68.0 ns	29.6 a
Bee-isolated (enclosed trees)	23.4 b	2.1 b	0.99 b	0.6 b	66.6	12.4 b
Significance level	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	*0.8218*	***<0.0001***
LSD 5%	*4.79*	*0.99*	*0.46*	*0.49*	-	*5.29*

Table 2.2. Effect of commercial beehives on the individual kernel weight, length, width, thickness, pellicle colour and roughness of 'Independence' almond trees at Groenrivier, Riebeek West (2019/2020). Means, within each column, with the same letter are not significantly different. ns = no significant difference.

Treatment	Individual kernel weight (g)	Kernel length (mm)	Kernel width (mm)	Kernel thickness (mm)	Pellicle colour	Kernel roughness
Bee pollinated (open trees)	1.0 b	22.9 b	11.4 b	8.2 ns	2.4 ns	1.7 ns
Bee-isolated (enclosed trees)	1.3 a	25.1 a	12.6 a	8.2	2.3	1.6
Significance level	***<0.0001***	***<0.0001***	***<0.0001***	*0.7260*	*0.1876*	*0.1102*
LSD 5%	*0.09*	*0.52*	*0.38*	-	-	-

Table 2.3. Effect of commercial beehives on the percentage double and shrivelled kernel of 'Independence' almond trees at Groenrivier, Riebeek West (2019/2020). Means, within each column, with the same letter are not significantly different. ns = no significant difference.

Treatment	Defects	
	Percentage double	Percentage shrivelled
Bee pollinated (open trees)	0.2 ns	0.7 ns
Bee-isolated (enclosed trees)	0.4	1.3
Significance level	*0.5399*	*0.2582*
LSD 5%	-	-

Table 3. Effect of commercial beehives on fruit set of 'Independence' almond trees at Groenrivier, Riebeek West (2020/2021). ns = no significant difference.

Treatment	Percentage fruit set	
	Trial site A	Trial site B
Bee pollinated (open branches)	25.5 a	32.7 ns
Bee-isolated (paper bag branches)	6.0 b	21.3
Significance level	***0.0021***	*0.0984*
LSD 5%	*11.75*	-

Table 4.1. Effect of hand pollination with 'Independence' flowers and 'Nonpareil' flowers on the percentage fruit set, dry weight, in-shell weight and kernel weight of branches, as well as percentage kernel, yield efficiency and branch nut density of 'Independence' almonds at Hex Poort, Worcester, Western Cape (2019/2020). ns = no significant difference.

Treatment	Percentage fruit set	Yield per branch			Percentage kernel	Branch yield efficiency ($g.cm^{-2}$)	Branch nut density (number of nuts per cm^2)
		Dry weight (g)	In-shell weight (g)	Kernel weight (g)			
Untreated control	26.33 ns	142.7 ns	70.3 ns	45.0 ns	64.27 ns	2.059 ns	2.351 ns
'Independence' x 'Independence'	29.46	139.0	67.6	44.0	65.27	2.175	2.399
'Independence' x 'Nonpareil'	28.01	120.3	61.1	39.2	64.23	2.024	2.378
Significance level	*0.6382*	*0.4581*	*0.5953*	*0.5750*	*0.6019*	*0.8587*	*0.9903*
LSD 5%	-	-	-	-	-	-	-

Table 4.2. Effect of hand pollination with 'Independence' flowers, as well as 'Nonpareil' flowers on the flower weight, length, width, thickness, colour and roughness of individual kernels, as well as, and shrivelled kernels of 'Independence' almonds at Hex Poort, Worcester, Western Cape (2019/2020). ns = no significant difference.

Treatment	Individual kernel						Defects
	Weight (g)	Length (mm)	Width (mm)	Thickness (mm)	Pellicle Colour	Roughness	Percentage Shrivelled
Untreated control	0.904 ns	22.265 ns	11.421 ns	7.614 ns	2.355 ns	1.574 ns	0.48 ns
'Independence' x 'Independence'	0.929	22.820	11.636	7.536	2.408	1.647	0.49
'Independence' x 'Nonpareil'	0.889	22.312	11.315	7.408	2.342	1.595	0.49
Significance level	*0.4604*	*0.0735*	*0.2025*	*0.3261*	*0.7057*	*0.3299*	*0.9990*
LSD 5%	-	-	-	-	-	-	-

Table 5. Effect of hand pollination with 'Independence' flowers, as well as 'Nonpareil' flowers on the percentage fruit set of 'Independence' almond trees at Hex Poort, Worcester (2020/2021). Means, within each column, with the same letter are not significantly different. ns = no significant difference.

Treatment	Percentage fruit set
Untreated control	48.81 ns
'Independence' x 'Independence'	52.62
'Independence' x 'Nonpareil'	49.34
Significance level	*0.7906*
LSD 5%	-

GENERAL DISCUSSION AND CONCLUSION

In light of seasonal progression throughout autumn and winter, changes in environmental conditions such as shorter photoperiods and lower minimum temperatures, lead to growth cessation in deciduous fruit trees, cueing the onset of dormancy induction (Beauvieux et al., 2018; Campoy et al., 2011a). The majority of dormancy research has focused on low temperatures for endodormancy development and physiological synchronization in deciduous fruit trees (Beauvieux et al., 2018; Crabbé and Barnola, 1996; Cook and Jacobs, 2000). Dormancy progression and bud break patterns in 'Independence' almond trees depicted low levels of dormancy. This is congruent with work done on other stone fruit cultivars under South African conditions (Cook, 2010; Campoy et al., 2011b). Great variation was shown among the different orchards in timing and development of dormancy induction and maximum dormancy levels, with chill accumulation, according to various chill models, generally starting after maximum dormancy was reached. However, dormancy release seemed to be more conforming among the different orchards showing a higher similarity in endodormancy breaking dates. Faust et al (1997) suggested that several factors, such as hormones, water content within dorman buds and anabolic potential of buds, as well as the membrane changes during dormancy, govern the gradual transition of paradormancy into endodormancy, complicating the process of dormancy induction. Once these factors have served their biological purpose, maximum dormancy is reached and physiological synchronization within the buds result in a more comparable manner through dormancy release. 'Independence' almond trees seem to progress through endodormancy, irrespective of chill accumulation, while being more reliant on heat accumulation for dormancy release and successful bud break.

The significant differences shown for chill requirement among the 'Independence' orchards indicate an interaction between the environmental and genetic factors regulating dormancy progression. Campoy et al. (2011c) emphasized that chill models do not account for physiological or functional biological processes within trees and can therefore not explain dormancy progression in full. Future studies should therefore include the synchronization of biological processes, as described by Andreini et al. (2012), when investigating the effect of chill accumulation and dormancy progression. The conceptual framework proposed by Fadón et al. (2020) serves as a wholistic approach to better understand the fundamental principles of dormancy progression. A greater reliance on heat requirement for dormancy release in 'Independence' also necessitates elucidation of when physiologically relevant heat accumulation starts, given that Campoy et al. (2011b) suggested a simultaneous accumulation

of both chill and heat units in apricot. Furthermore, inadequacies of forcing experiments could have contributed to the variability in results obtained (Dennis, 2003). Terblanche et al. (1979) speculated that an interaction exits between carbohydrate reserves and dormancy release in the case of apple. Almond is a novel crop for South African growers and a lack of practical experience could have led to differences in the nutritional status among the orchards sampled, contributing to the variation seen in dormancy progression. Our trials indicated that 'Independence' is suited for production in most parts of the Western Cape, however, orchards could not be compared in their suitability. Future studies should focus on multiple trial sites within one production region to elucidate whether the variability in dormancy progression seen in our work can be ascribed to regional factors, or whether management practices can have a significant influence. This would now be possible given the increase in plantings in recent times. Furthermore, studies should include flower density and yield productivity to enable direct comparison between orchards, as Tombesi et al. (2016) have indicated a higher correlation between flower abundance and crop load in almond, compared to fruit set.

In the process of determining the efficacy of various rest breaking treatments in 'Independence' almond trees, no effect was found in reproductive bud break, fruit set or post-harvest quality parameters. When considering the low levels of endodormancy and chill requirement (CR) we found in our forcing trials, as well as speculations of a lower CR in floral buds, compared to vegetative buds (Saure, 1985), this was not surprising. Interestingly, rest breaking treatments did affect the vegetative bud break by advancing its onset, while enhancing the number of vegetative buds to break in some cases when reproductive bud break was at its maximum. Although this greater overlap between reproductive and vegetative bud break created more competition between meristems, it did not disadvantage the total percentage reproductive bud break, fruit set or yield efficiency, when compared to the untreated control. Furthermore, studies have shown that newly formed peach leaves transform from a sink to a source approximately seven to ten days after vegetative bud break (Marchi et al. 2005). Advanced vegetative bud break (that was up to 10 - 20 days earlier) could, therefore, lead to earlier production of photosynthetic assimilates increasing the carbohydrate supply for reproductive development and fruit set, as well as the accumulation of reserves for bud break in the following year.

The notion that almonds primarily bear on spurs (Tombesi et al., 2016) was also evident in our fruit set results. This could benefit future yield as the growth index results from this study showed an increase in spur formation in trees receiving rest breaking treatments,

however, this should be evaluated in long term studies. The RBAs evaluated, did not affect the overall vegetative growth index of 'Independence' almond trees. However, in an orchard subjected to environmental stresses, increased spur formation can compromise the production of short shoots. Rest breaking treatments containing oil proved efficient in advancing the onset of vegetative bud break and enhancing spur production in 'Independence' almond trees, with 0.5% hydrogen cyanamide in combination with 2% mineral oil generally showing the best results. The trade-off between short shoot growth in favour of spur production under resource limited conditions may be a positive attribute to high density almond plantings. Stress induced by inter-tree competition in these orchards could lead to a higher spur production and possibly increase long-term yield potential. However, yield and post-harvest results for this study is based on data from a single season. To accurately determine the effect of RBAs in increasing the effective bearing surface and possible crop load, longer term studies are needed. It should, be noted that increased spur formation would require sufficient renewal pruning, given that almond spurs have a productive lifespan of approximately five years (Weinbaum and Spiegel-Roy, 1985).

Results from our study indicated better quality flowers borne on spurs of 'Independence' almond trees, as indicated by flower weight. This phenomenon, together with reduced vegetative growth competition, is likely the reason for a higher fruit set percentage on spurs, compared to other bearing positions, as seen in our results. The length of the style exceeded that of the stamen in all flowers, irrespective of bearing position, confirming the epistigmatic nature of the flowers from this self-compatible cultivar. Results of the style to stamen ratio in our study is congruent with that in eleven of the sixteen self-compatible almond cultivars studied by Kumar and Kumar (2000). The presence of 'Nonpareil' as a compatible cross-pollinator did not influence the percentage fruit set, yield efficiency or post-harvest quality parameters, which is congruent with results from Dicenta et al. (2002). Establishing single-cultivar orchards could therefore present practical and financial benefits for almond producers. Results in this study indicated that 'Independence' almond trees were capable of having an average fruit set percentage of more than 20% in the complete absence of bees as pollinators. The autogamic capacity expressed in epistigmatic 'Independence' flowers correspond with work done by Godini et al. (1992). These authers indicated no relationship between the fruit set ability and stigma/anther spatial ratio in self-compatible almond cultivars, with regards to unassisted self-pollination. However, these authors found a considerable increase in fruit set percentage, following assisted self-pollination. This was also the case in

our results, where trees subject to commercial beehives showed a substantially higher average fruit set percentage, compared to the exclusion of bees as pollinators. Our results indicate a reduced dependency on pollen vectors, however, 'Independence' has not been rendered pollinator-independent when pursuing maximum yield potential. Future studies should investigate the relationship between the number of bee colonies and total tree volume, to determine an economically efficient colony density to ensure optimal yield in self-compatible almond cultivars, such as 'Independence'. Furthermore, when conducting studies on the dependency of an almond cultivar on bees as pollinators, the work of Knight et al. (2006) should be taken into account to avoid an over-estimation of pollinator-dependency in the studied cultivar.

Our results have indicated a high suitability of 'Independence' almond trees to environmental conditions in the Western Cape, given its intrinsic low CR in combination with a higher HR. Dormancy progression and successful bud break was achieved, even in the absence of artificial RBAs, which has become standard commercial practice for deciduous fruit production in South Africa. 'Independence' is not reliant on a compatible cross-pollinator, however, pollen vectors are still required to achieve maximum yield potential.

Literature Cited

Andreini. L., R. Viti, S. Bartolini, D. Ruiz, J. Egea, and J.A. Campoy. 2012. The relationship between xylem differentiation and dormancy evolution in apricot flower buds (*Prunus armeniaca* L.): the influence of environmental conditions in two Mediterranean areas. Trees 26:919-928.

Beauvieux, R., R. Wenden, and E. Dirlewanger. 2018. Bud dormancy in perennial fruit tree species: a pivotal role for oxidative cue. Frontiers Plant Sci. 9(657):1-13.

Cook, N.C. 2010. Final report: Development and testing of a new chilling model for low chill conditions. Hortgro, Paarl, South Africa

Cook, N.C. and G. Jacobs. 2000. Progression of apple (*Malus* x *domestica* Borkh.) bud dormancy in two mild winter climates. J. Hort. Sci. Biotechnol. 75(2)233-236.

Campoy, J.A., D. Ruiz, N. Cook, L. Allderman, and J. Egea. 2011a. Clinal variation of dormancy progression in apricot. S. Afr. J. Bot. 77:618-630.

Campoy, J.A., D. Ruiz, N. Cook, L. Allderman, and J. Egea. 2011b. High temperatures and time to budbreak in low chill apricot 'Palsteyn'. Towards a better understanding of chill and heat requirements fulfilment. Sci. Hort. 129:649-655.

Campoy, J.A., D. Ruiz, and J. Egea. 2011c. Dormancy in temperate fruit trees in a global warming context: A review. Sci. Hort. 130:357-372.

Crabbé, J. and P. Barnola. 1996. A new conceptual approach to bud dormancy in woody plants, 83-113. In: G.A. Lang (ed.). Plant dormancy. BABI, OXON, UK.

Dennis F. 2003. Problems in standardizing methods for evaluating the chilling requirements for the breaking of dormancy in buds of woody plants. Hort Science 28 (3), 347-350.

Dicenta, F., E. Ortega, J.A. Cánovas, and J. Egea. 2002. Self-pollination vs. cross-pollination in almond: pollen tube growth, fruit set and fruit characteristics. Plant Breeding 121:163-167.

Fadón, E., E. Fernandez, H. Behn, and E. Luedeling. 2020. A conceptual framework for winter dormancy in deciduous trees. Agronomy 10(2):1-20.

Faust, M., A. Erez, L.J. Rowland, S.Y. Wang, and H.A. Norman. 1997. Bud dormancy in perennial fruit trees: physiological basis for dormancy induction, maintenance, and release. HortScience 32:623–629.

Godini, A., L. de Palma, and M. Palasciano. 1992. Role of self-pollination and reciprocal stigma/anther position on fruit set of eight self-compatible almonds. HortScience 27(8):887-889.

Knight, T.M., J.A. Steets, J.A., and T.L. Ashman. 2006. A quantitative synthesis of pollen supplementation experiments highlights the contribution of resource reallocation to estimates of pollen limitation. J. Amer. Soc. Hort. Sci. 93(2):271-277.

Kumar, K. and U. Kumar. 2000. Flower morphology versus self-compatibility in almond. Acta Hort. 522:97-102.

Marchi, S., L. Sebastiani, R. Gucci, and R. Tognetti. 2005. Sink-source transition in peach leaves during shoot development. J. Amer. Soc. Hort. Sci. 130(6):928-935.

Saure, M.C. 1985. Dormancy release in deciduous fruit trees. Hort. Rev. 7:239-300.

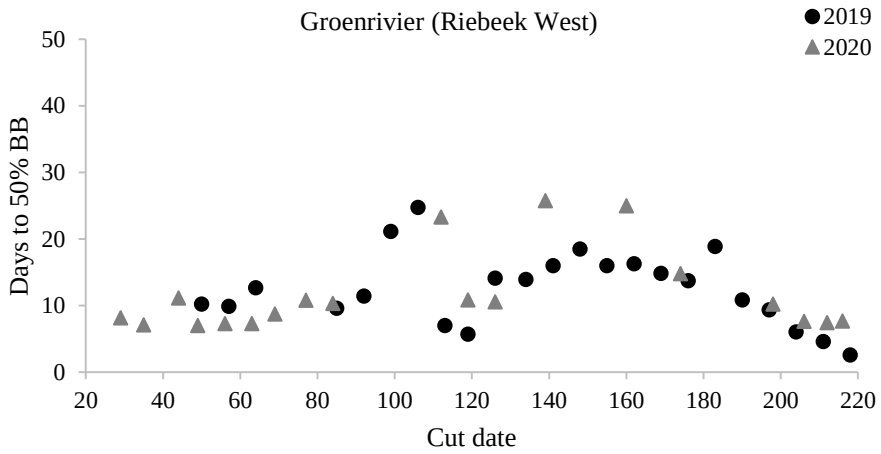
Terblanche, J.H., P.J.C. Stassen, I. Hesebeck, and D.K. Strydom. 1979. Effect of autumn nitrogen nutrition and a winter rest-breaking spray on the growth, development and chemical composition of young 'Golden Delicious' apple trees grown in sand culture. Sci Hort. 10:37-48.

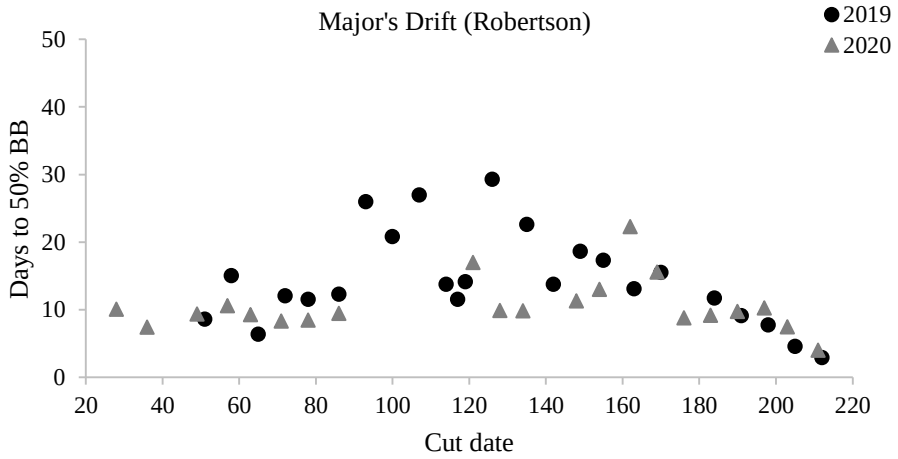
Tombesi, S., B.D. Lampinen, S. Metcalf, and T.M. DeJong. 2016. Yield in almond is related more to the abundance of flowers than the relative number of flowers that set fruit. Calif. Agric. 71(2):68-74.

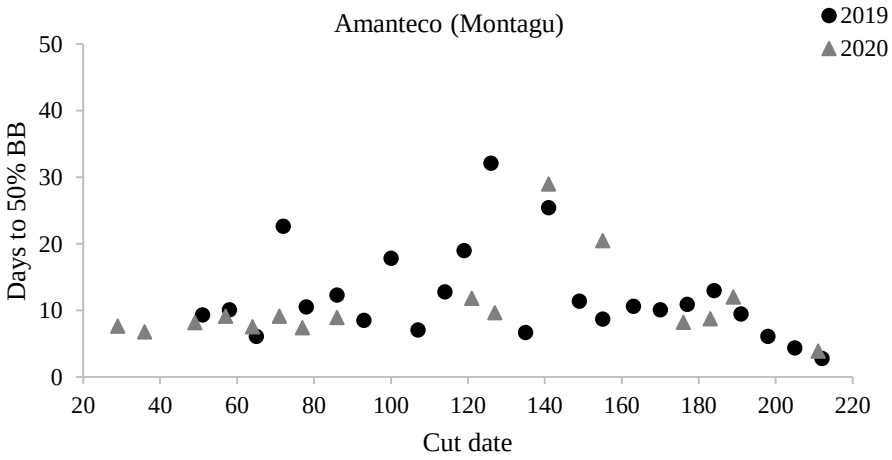
Weinbaum, S.A. and P. Spiegel-Roy. 1985. The almond, 139-146. In: A.H. Harvey (ed.). Handbook of flowering. CRC press inc., FL, USA.

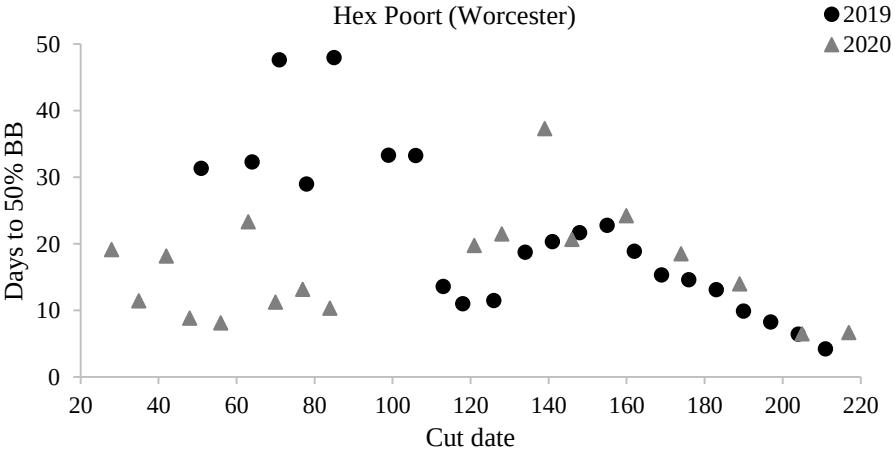
APPENDIX A:

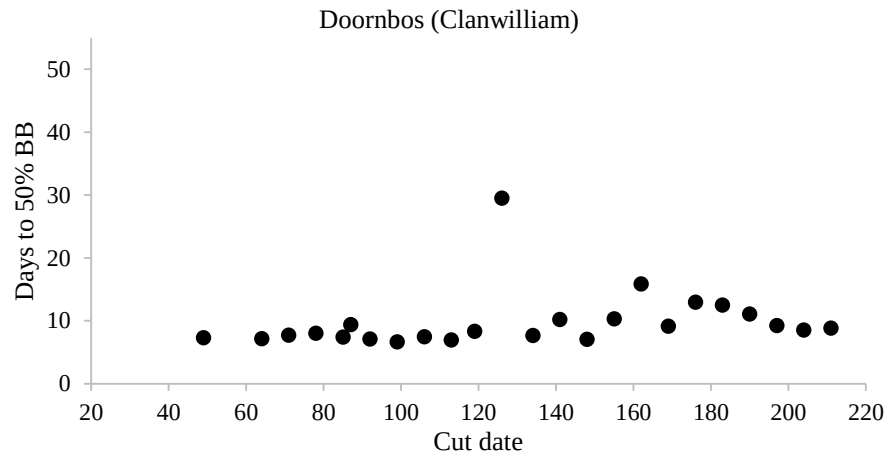
Individual scatter plots of the eight commercial orchards representing their average dormancy levels (days to 50% bud break) for each of the collection dates indicated as day of the year, during the 2019 season, as well as for those during the 2020 season. BB = bud break

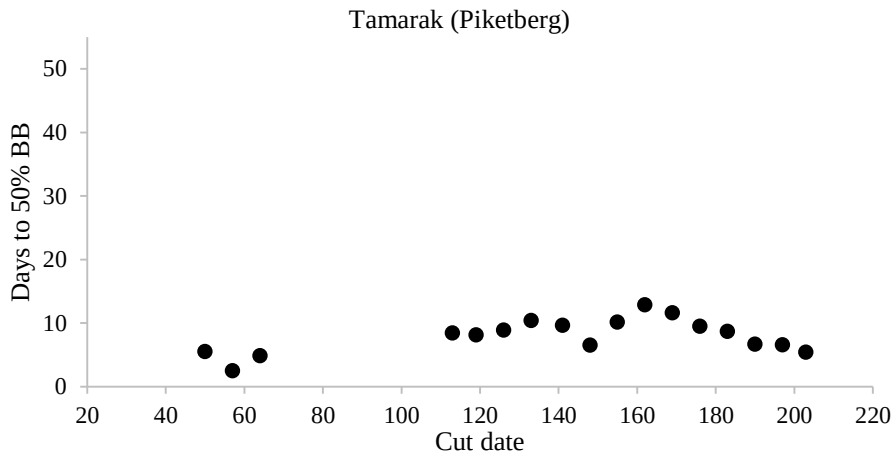


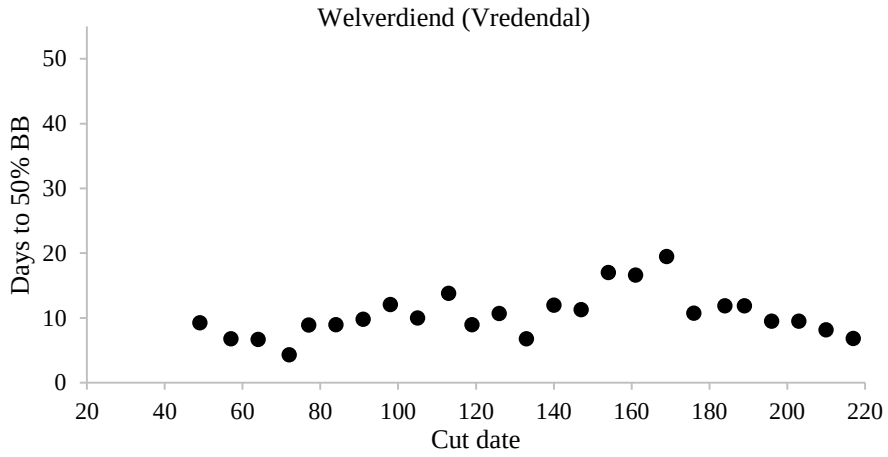


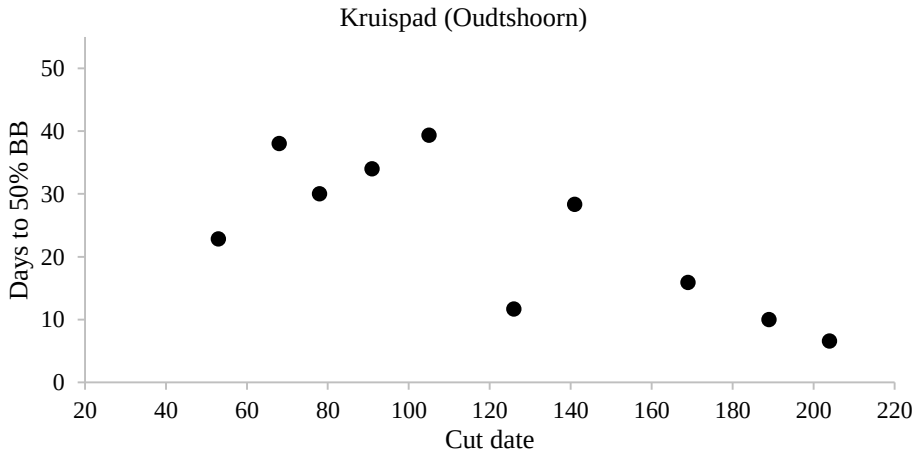






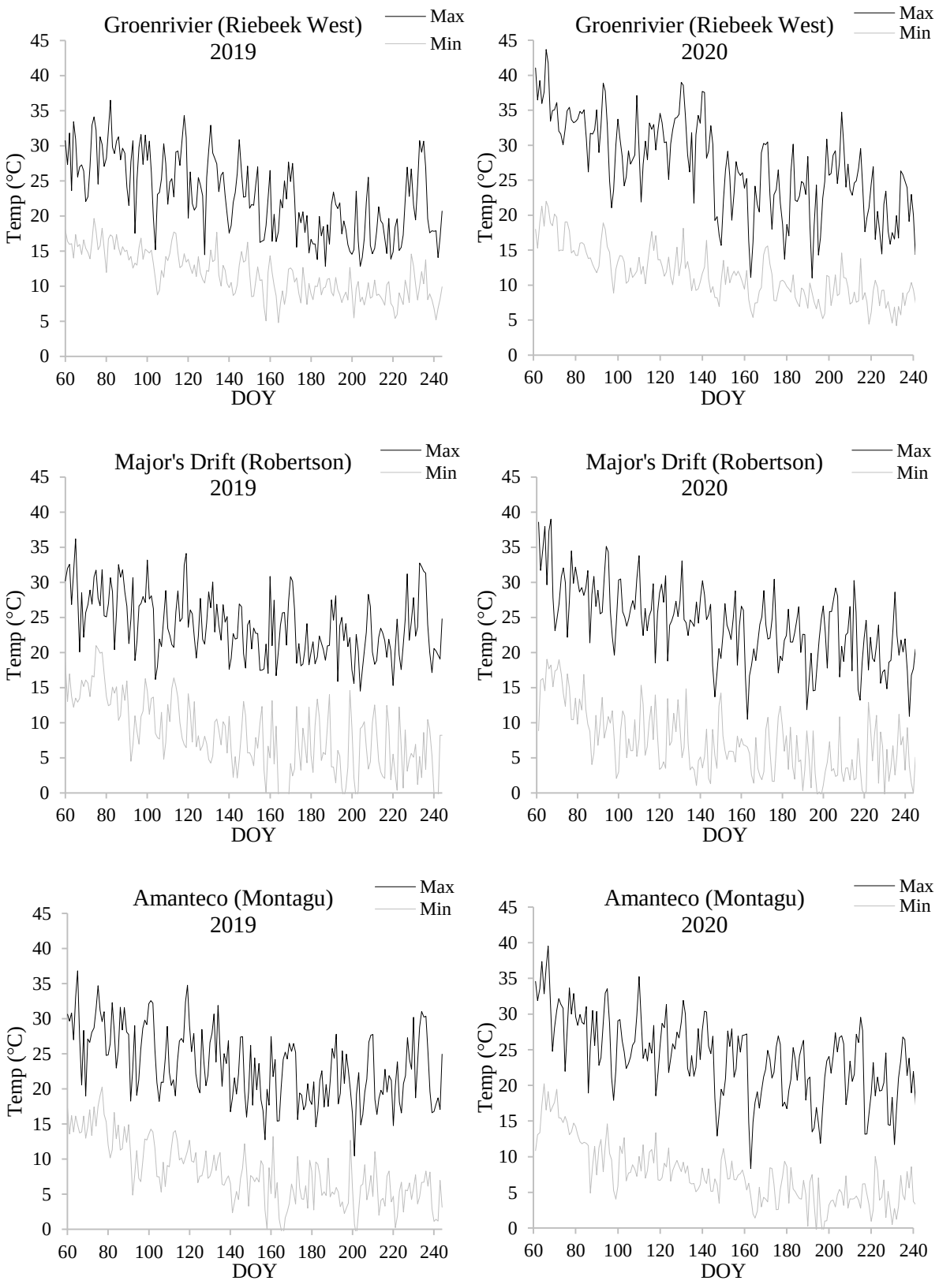


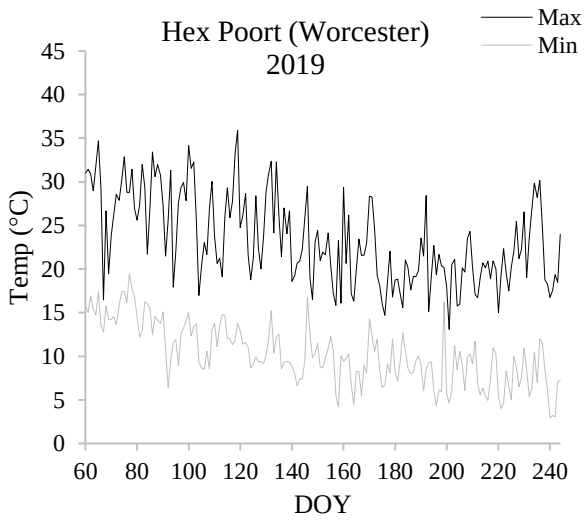


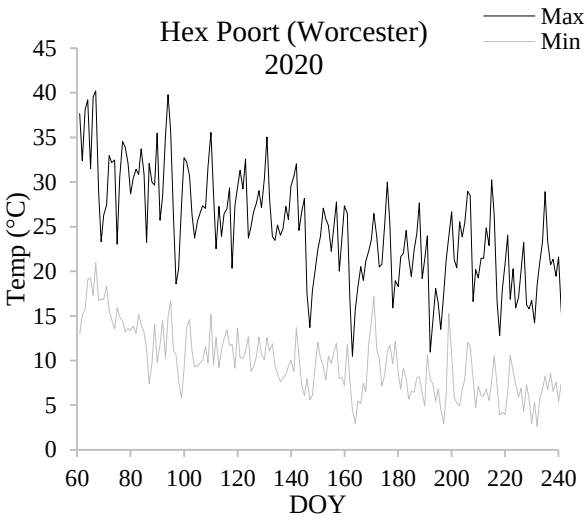


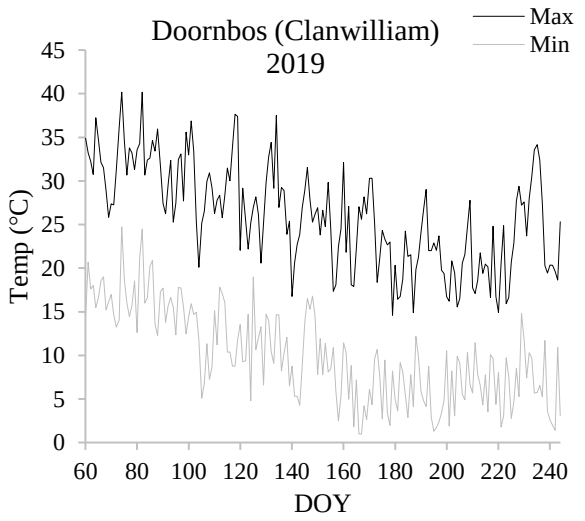
APPENDIX B:

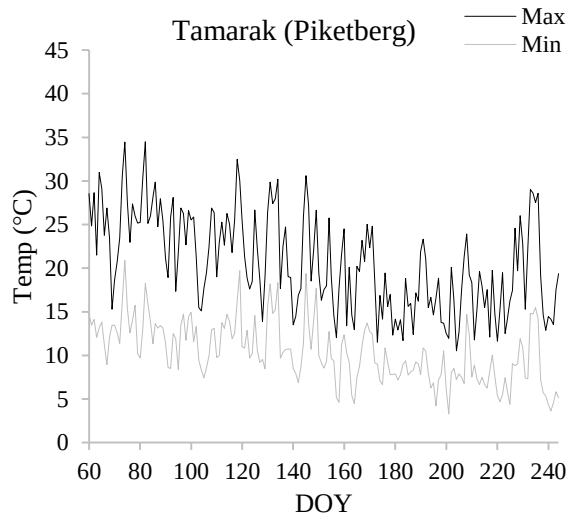
The daily maximum and minimum temperatures (°C) for each of the commercial orchards during the 2019 and 2020 season. DOY = day of year

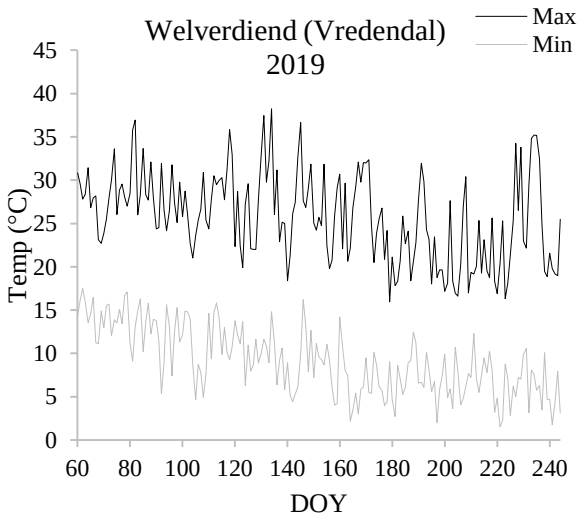


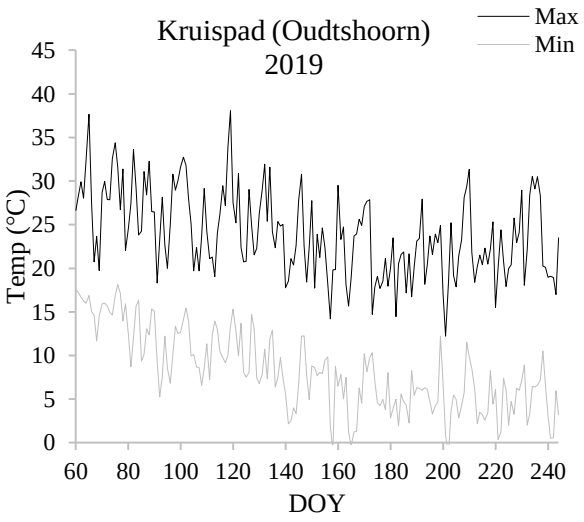






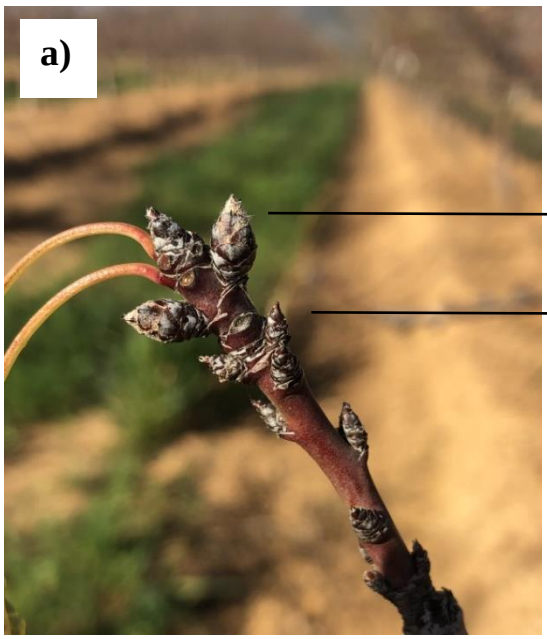


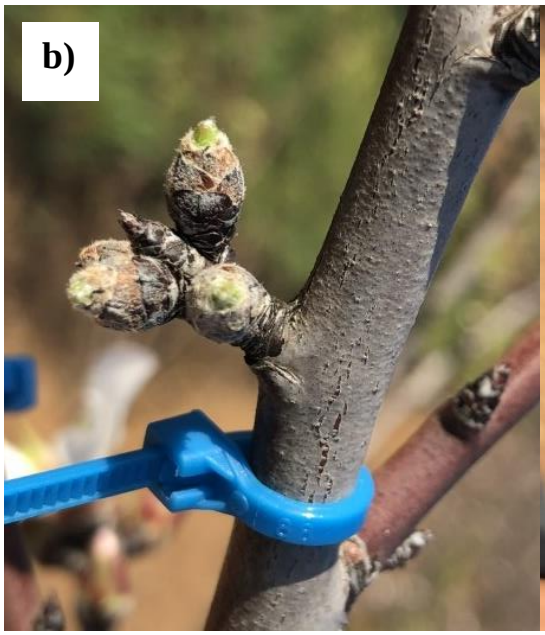




APPENDIX C:

Various stages of growth recorded in ‘Independence’ almond buds, namely a) bud swell, b) reproductive bud break (visible calyx) and c) vegetative bud break (green tip). Photos by T. du Toit.





APPENDIX D:

The orchard in the a) Worcester region had difficulty with weed management, especially on the ridging, compared to the orchard in the b) Riebeek West region. This could increase competition for available water and nutrients, possibly having a negatively impact on vegetative and reproductive bud break and growth. Photos by T. du Toit.